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[Continued on next page]

(54) Title: VACCINE

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1  GGGCCGGTGCCAAGATCTGTGCTCTCCCTCTGACCCCTTAGGGAGCTCATTGAGGAGCTG  60
   G P V P R S V S L P L T L R E L I E E L
61  GTCACATCACACAAGACCAGACTCCCTGTGCAACGGCAGCATGGTATGGAGTGGAC  120
   V N I T Q D Q T P L C N G S M V W S V D
121 CTGGCCGGTGGGGTACTGTGTCAGCCCTGGAAATCCCTGACCAACATCTCCAATTGCAAT  180
   L A A G G Y C A A L E S L T M I S N C H
181 GCCATCGAAGACCCAGAGGATGCTGGGCGGACTCTGTAACCGCAAGGCCCCCACTACG  240
   A I E K T Q R M L G G L C N R K A P T T
241 GTCTCAGCCTCCCGATACCAAAATCGAGGTGGCCAGTTTGTAAAGGACCTGCTCAGC  300
   V S S L P D T K I E V A Q F V K D L L S
301 TACACAAAGCAACTGTTTGGCCACGGCCCTCTTAA  336
   Y T K Q L F R H G P P *

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(57) Abstract: The present invention relates to an isolated polypeptide useful for immunisation against self-antigens. In particular the invention relates to a self-protein that is capable of raising auto-antibodies when administered in vivo. The invention particularly relates to rendering human cytokines immunogenic in humans. The invention further relates to pharmaceutical compositions comprising such compounds and their use in medicine and to methods for their production.

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-23  -----+-----+  0
      NGLTSQILLPPLPFLACAGNFVHG
1    -----+-----+  24
      HKCDKQHLRRIIGILNEVTGKTL
25   -----+-----+  48
      CTBLTVTDI PAASKVTTESELVCR
49   -----+-----+  72
      ABKVLRIPIYLKHEKDTRELGATAK
73   -----+-----+  96
      NSSLVMEQLRQLFRAPRCLDGLNSC
97   -----+-----+  120
      FVKRANQSSIKDFLESLSKSIHQND
121  -----  126
      YSKCSS

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For two-letter codes and other abbreviations, refer to the "Guidance Notes on Codes and Abbreviations" appearing at the beginning of each regular issue of the PCT Gazette.

Vaccine

The present invention relates to an isolated polypeptide useful for immunisation against self-antigens. In particular the invention relates to a self-protein that is
5 capable of raising auto-antibodies when administered in vivo. The invention particularly relates to rendering human cytokines immunogenic in humans. The invention further relates to pharmaceutical compositions comprising such compounds and their use in medicine and to methods for their production.

10 Background of the invention

Asthma is a chronic lung disease, caused by inflammation of the lower airways and is characterised by recurrent breathing problems. Airways of patients are sensitive and swollen or inflamed to some degree all the time, even when there are no
15 symptoms. Inflammation results in narrowing of the airways and reduces the flow of air in and out of the lungs, making breathing difficult and leading to wheezing, chest tightness and coughing. Asthma is triggered by super-sensitivity towards allergens (e.g. dust mites, pollens, moulds), irritants (e.g. smoke, fumes, strong odours), respiratory infections, exercise and dry weather. The triggers irritate the airways and
20 the lining of the airways swell to become even more inflamed, mucus then clogs up the airways and the muscles around the airways tighten up until breathing becomes difficult and stressful and asthma symptoms appear.

COPD is an umbrella term to describe diseases of the respiratory tract, which shows
25 similar symptoms to asthma and is treated with the same drugs. COPD is characterised by a chronic, progressive and largely irreversible airflow obstruction. The contribution of the individual to the course of the disease is unknown, but smoking cigarettes is thought to cause 90% of the cases. Symptoms include coughing, chronic bronchitis, breathlessness and respiratory infections. Ultimately
30 the disease will lead to severe disability and death.

As a result of the various problems associated with the production, administration and tolerance of monoclonal antibodies there is an increased focus on methods of instructing the patient's own immune system to generate endogenous antibodies of the appropriate specificity by means of vaccination. However, mammals do not
5 generally have high-titre antibodies against self-proteins present in serum, as the immune system contains homeostatic mechanisms to prevent their formation. The importance of these tolerance mechanisms is illustrated by diseases like myasthenia gravis, in which auto-antibodies directed to the nicotinic acetylcholine receptor of skeletal muscle cause weakness and fatigue (Drachman, 1994, *N Engl J Med*
10 330:1797-1810). There is therefore a need for a vaccine approach which is able to circumvent antibody tolerance mechanisms without inducing auto-antibody-mediated pathology.

A number of techniques have been designed with the aim of breaking B cell
15 tolerance without necessarily inducing unacceptable autoimmune toxicity. However, all have significant drawbacks.

One technique involves chemically cross-linking either the self-protein (or peptides derived from it) to a highly immunogenic carrier protein, such as keyhole limpet
20 haemocyanin (Antibodies: A laboratory manual" Harlow, E and Lane D. 1988. Cold Spring Harbor Press). This approach is a variant of the widely used hapten-carrier system for raising antibodies to poorly immunogenic targets, such as low-molecular weight chemical compounds. However, the process of chemical conjugation can destroy potentially valuable epitopes, and much of the evoked antibody response is
25 directed at the carrier protein. Furthermore this approach is only applicable to protein vaccination, and is not compatible with nucleic acid immunogens.

A variant on the carrier protein technique involves the construction of a gene encoding a fusion protein comprising both carrier protein (for example hepatitis B
30 core protein) and self-protein (The core antigen of hepatitis B virus as a carrier for immunogenic peptides", *Biological Chemistry*. 380(3):277-83, 1999). The fusion

gene may be administered directly as part of a nucleic acid vaccine. Alternatively, it may be expressed in a suitable host cell *in vitro*, the gene product purified and then delivered as a conventional vaccine, with or without an adjuvant. However, fusing a large carrier protein to the self-protein can constrain or distort the self-protein's conformation, reducing its efficiency in evoking antibodies cross-reactive with the native molecule. Also, like the traditional cross-linked carrier systems, much of the antibody response is directed to the carrier part of the fusion. Anti-carrier responses may limit the effectiveness of subsequent booster administrations of vaccine or increase the chance of allergic or anaphylactic reactions.

10

A more refined approach has been described by Dalum and colleagues wherein a single class II MHC-restricted epitope is inserted into the target molecule. They demonstrated the use of this method to induce antibodies to ubiquitin (Dalum et al, 1996, *J Immunol* 157:4796-4804; Dalum et al, 1997, *Mol Immunol* 34:1113-1120) and the cytokine TNF (Dalum et al, 1999, *Nature Biotech* 17:666-669). As a result, all T cell help must arise either from this single epitope or from junctional sequences. While this approach may work well in subjects possessing the appropriate MHC class II haplotype for which the vaccine was designed, or indeed those fortunate enough to have class II molecules capable of binding junctional epitopes, in any normal outbred population, such as those typical of humans, there will be a significant portion of the population for whom the vaccine will not work. Additionally, since the inserted epitope is typically from a quite unrelated protein, such as ovalbumin or lysozyme, it is likely that the additional sequence will to some degree interfere with the folding of the target protein, preventing the adoption of a fully native conformation of the target protein.

25

In contrast to all of the above, the present invention provides a multiplicity of potential T cell epitopes, yet retains the target molecule in a conformation close to the native form. These properties allow the vaccines of the present invention to be effective immunogens in complex outbred populations, such as those composed of human patients. These properties are achieved by rendering a mutation in a self-

30

protein to produce a sequence at that point which can be found in an analogous protein.

A number of recent papers have defined a critical role for the Th2 cytokine IL-13 in driving pathology in the ovalbumin model of allergic asthma (Wills-Karp et al, 1998; Grunig et al, 1998). In this work, mice previously sensitised to ovalbumin were injected with a soluble IL-13 receptor which binds and neutralises IL-13. Airway hyper-responsiveness to acetylcholine challenge was completely ablated in the treated group. Histological analysis revealed that treated mice had reversed the goblet-cell metaplasia seen in controls. In complementary experiments, lung IL-13 levels were raised by over-expression in a transgenic mouse or by installation of protein into the trachea in wild-type mice. In both settings, airway hyper-responsiveness, eosinophil invasion and increased mucus production were seen (Zhu et al, 1999). These data show that IL-13 activity is both necessary and sufficient to produce several of the major clinical and pathological features of allergic asthma in a well-validated model.

A vaccine capable of directing a neutralising response to IL-13 would therefore constitute a useful therapeutic for the treatment of allergic asthma in humans. It would also have application in the treatment of certain helminth infection-related disorders (Brombacher, 2000) and diseases where IL-13 production is implicated in fibrosis (Chiaramonte et al, 1999), such as chronic obstructive pulmonary disease. The present invention addresses this need.

The concepts and principles of the invention are thus set forth with respect to IL-13, but can be applied to any mammalian self-protein having an analogous protein in a second species.

Summary of the Invention

The present invention provides an isolated polypeptide which is at least 30% but less than 100% identical to a human protein which polypeptide

- 5 (a) contains at least one mutation which is characteristic of an analogous non human protein;
and b) is capable of raising antibodies in humans and
(c) is sufficiently structurally similar to the human protein that the antibodies bind to both the human protein and the polypeptide; and
10 (d) wherein the polypeptide is not an antibody.

Thus the invention provides in one embodiment; a protein having B cell epitopes from a mammalian self-antigen and a mutation that gives rise to a sequence of an analogous protein from a second mammalian species, such that the protein is able
15 to raise in the species from which the B-cell epitopes are derived, an immune response that recognises the native protein from which the B-cell epitopes are derived.

Preferably the sequence of the analogous protein is more than 5, more preferably
20 greater than 8 contiguous amino acids. Thus the protein of the present invention contains a sequence that is identical to the analogous sequence for at least 5, preferably at least 8 consecutive amino acids. In an alternative embodiment a protein is provided having B cell epitopes of a self protein which are grafted by substitution, into a framework of an analogous protein from a second mammalian
25 species such that the protein is able to raise in the species in which the B cell epitopes are derived an immune response that recognises the natural protein from which the B-cell epitopes are derived.

It will be appreciated that the protein of the present invention are not an antibody.

The immune response raised is preferably an antibody response, most preferably a neutralising antibody response.

In general the mutation is introduced preferably into the non-surface exposed region
5 of the molecule, such that surface exposed regions are conserved. Surface exposed regions are accessible to the immune-system and consequently often contain B-cell epitopes. Accordingly the present invention provides a protein comprising conserved surface exposed regions of a self protein, and a mutation introduced into the non-surface exposed region, said mutation giving rise to a sequence of an
10 analogous protein such that the protein is able to raise an immune response to the self-protein arises in the species from which the self-protein is derived.

The self protein is preferably a human protein, but can be a protein from any mammal in which it is desired to raise an auto immune response to. The immune
15 response is preferably specific to the native protein and immunogen of the invention. That is having minimal cross-reactivity or neutralising capacity with respect to other self proteins.

The self antigen is preferably a cytokine, more preferably a 4 helical cytokine, more
20 preferably IL-4 or IL-13, most preferably IL-13. Thus in a preferred embodiment of the present invention there is provided a chimaeric protein comprising B cell epitopes from Human IL-13 presented in a murine IL-13 back bone. Such a construct is capable of raising a specific anti IL-13 antibody response in humans. Such a construct is shown in figure 9 (seq: ID No 21 and 22). Similarly an IL-4
25 construct comprising human IL-surface regions and murine framework is presented in figure 13 (Seq ID: No 25).

The invention also provides:

- an expression vector which comprises a polynucleotide of the invention and
30 which is capable of expressing a polypeptide of the invention;
- a host cell comprising an expression vector of the invention;

a method of producing a polypeptide of the invention which method comprises maintaining a host cell of the invention under conditions suitable for obtaining expression of the polypeptide and isolating the said polypeptide:

a vaccine composition comprising a polypeptide or polynucleotide of the invention
5 and a pharmaceutically acceptable carrier.

In another aspect, the invention provides a method for the design and preparation of a polypeptide according to the invention which method comprises:

1. identification of one or more regions of a self, typically human, protein against
10 which an antibody response is desired.
2. identification of the amino-acid sequence of the self protein.
3. identification of the amino-acid sequence of an analogous protein
construction by recombinant DNA techniques of a chimaeric molecule containing
at least one target region identified in step 1, whose amino-acid sequence is taken
15 from the sequence identified in step 2, and
sufficient amino-acids from the sequence(s) identified in step 3 to enable the
resulting protein to fold into a shape similar to that the self protein such that the
mutated protein can raise an immune response that recognises the self protein.

20 **Description of Figures**

GST = glutathione S-transferase, rmlL-13 = recombinant mouse IL-13, rhIL-13 =
recombinant human IL-13, cIL-13 = chimaeric IL-13

Figure 1. Sequence of mouse chimaeric IL-13 vaccine construct. Underlined
25 aminoacid symbols denote sequence human IL-13, unmodified symbols are from
murine IL-13.

Figure 2. Analysis of GST cIL-13 by 4-20% Tris-glycine SDS-PAGE gel (Novex),
stained for total protein with Coomassie Blue.

30

Figure 3. Western blot analysis of GST-cIL-13.

Figure 4. ELISA analysis of cIL-13 and GST-cIL-13 interaction with anti-mIL-13 polyclonal antibody, anti-hIL-13 polyclonal antibody and anti-GST polyclonal antibody.

5 Figure 5. ELISA analysis of the interaction of cIL-13 and GST-cIL-13 with the mIL-13 receptors, mIL-13R α 1 and mIL-13R α 2.

Figure 6. Anti-phospho-STAT6 Western blot of A549 lysates.

10 Figure 7. Antibody responses induced by immunisation with GST-cIL-13 (mouse F5) or cIL-13 (mouse E5).

Figure 8. Anti-phospho-STAT6 Western blot analysis of A549 lysates.

15 Figure 9 Chimaeric IL-13 vaccine for use in humans. Underlined aminoacid symbols denote sequence found in murine IL-13, unmodified symbols are from human IL-13.

Figure 10. Anti-mouse IL-13 antibody profiles follow administration of cIL-13 in combination with various adjuvants.

20

Figure 11. Serum neutralisation capacity of mice following administration of cIL-13.

Figure 12. Alternative cIL-13 for use as a mouse immunogen.

25

Figure 13. Chimaeric IL-4 for use in human anti IL-4 vaccine.

Detailed description of the invention

30 Throughout this specification and the appended claims, unless the context requires otherwise, the words "comprise" and "include" or variations such as "comprising",

"comprises", "including", "includes" etc., are to be construed inclusively, that is, use of these words will imply the possible inclusion of integers or elements not specifically recited.

5 As described herein, the present invention relates isolated polypeptides and isolated polynucleotides. In the context of this invention the term "isolated" is intended to convey that the polypeptide or polynucleotide is not in its native state, insofar as it has been purified at least to some extent or has been synthetically produced, for example by recombinant methods, or mechanical synthesis. The term "isolated"
10 therefore includes the possibility of the polypeptides or polynucleotides being in combination with other biological or non-biological material, such as cells, suspensions of cells or cell fragments, proteins, peptides, expression vectors, organic or inorganic solvents, or other materials where appropriate, but excludes the situation where the polynucleotide is in a state as found in nature.

15

An advantage of the invention is that the polypeptide of the invention contains regions of the self, eg human protein against which an antibody response is desired, in association with regions characteristic of an analogous protein which are sufficiently different to the human protein to provide excellent T cell help, but yet are
20 optimised by evolution to fold into a shape highly similar to the human protein. This allows antibodies to be raised that recognise the self antigen. Typically, the immune response raised includes the raising of a neutralising antibody response.

The human protein according to the invention may be a full length protein encoded
25 by the human genome or a domain or sub-unit of a full length protein encoded by the human genome. Where it is desired to raise neutralising antibodies against a functional domain of the self antigen – or a receptor binding domain a chimaeric antigen involving only these regions may be prepared. Thus the exposed region of such a domain, or the B cell epitopes of such a domain are conserved and mutation
30 of an analogues protein is introduced in the non-B cell epitope or surface exposed domains.

The term 'protein' is intended to include, for example, shorter sequences of amino acid residues which may be referred to as peptides, such as neuropeptides. The human protein will typically be the subject of post-translational modification such as glycosylation, proteolytic cleavage, phosphorylation, and others well known to those skilled in the field. The human protein is preferably a cytokine, a hormone, a growth factor or an extracellular protein, more preferably a 4-helical cytokine, most preferably IL-13. Cytokines include, for example, IL1, IL2, IL3, IL-4, IL5, IL6, IL7, IL8, IL9, IL10, IL11, IL12, IL13, IL14, IL15, IL16, IL17, IL18, IL20, IL21, IL25, TNF, TGF, GMCSF, MCSF and OSM. 4-helical cytokines include IL2, IL3, IL-4, IL5, IL13, GMCSF and MCSF. Hormones include, for example, luteinising hormone (LH), follicle stimulating hormone (FSH), chorionic gonadotropin (CG), VGF, GHrelin, agouti, agouti related protein and neuropeptide Y. Growth factors include, for example, VEGF. Extracellular proteins include, for example, APP or B-amyloid.

15 An analogous protein is one which is orthologous or paralogous to the self-protein, eg human protein, wherein an orthologous protein can be traced by descent to a common ancestor of the different organisms and is therefore likely to perform similar conserved functions in the different organisms. Thus an orthologous gene means genes which are so similar in sequence they have originated from a single ancestral gene and thus are an equivalent gene in a different species and have evolved from a common ancestor by specification. In particular in humans the orthologous protein is a structurally equivalent molecule in a non human mammal. A paralogous protein is one which appears in more than one copy in a given organism by a duplication event (Venter, Science; 1336, vol 291; 2001), that is homologous sequence (sharing a common evolutionary ancestors) that have diversified by gene duplication. Preferably the analogous protein is an orthologue. An orthologous protein will typically have the same name as the human protein and will typically perform the same function, for example murine IL-13 is the orthologue to human IL-13. The analogous protein is typically mammalian or avian, for example, bovine, ovine, rodent, such as murine, porcine, simian, feline, canine or human. Preferably the analogous protein is 25 murine. Thus in the context of the present invention, Murine IL-13 is an analogous

(and orthologous) protein to human IL-13. Similarly simian IL-4 is an analogous (and orthologous) protein to human IL-4.

The polypeptide of the invention preferably comprises 2, 3, 4, 5, 6, 7, 8, 9, 10, 11 or
5 more mutations characteristic of an analogous protein. More preferably the polypeptide comprises at least three mutations. Each mutation may be characteristic of the same or different analogous proteins. Thus a first mutation might be characteristic of a murine analogue and a second mutation might be characteristic of a simian analogue. According to one feature, the polypeptide
10 comprises at least three mutations, where each mutation is characteristic of a different analogue. Preferably, however, each mutation is characteristic of the same analogue. A mutation is a change in the amino acid sequence of the protein and includes, for example, deletions, insertions and substitutions. Preferably the mutation is a substitution. Preferably more than one amino acids are replaced in
15 each non-surfaced exposed region.

A mutation which is characteristic of an analogous protein is one which results in the sequence of the human protein being closer in identity to the sequence of the analogous protein after the mutation has been made to the human protein. For
20 example when the human sequence is ProProArgVal and the murine analogue has the sequence ProProTyrVal, a mutation characteristic of the analogous protein is to substitute Arg for Tyr. Preferably the mutation is not made in residues which are surface residues in native folded active protein in aqueous solution under physiological conditions. These surface residues particular those forming loop
25 structures are often B cell epitopes and it is preferred that all of these regions are conserved. The mutations thus introduced have the function of breaking the tolerance of the self-protein and being immunogenic in the species that the non-mutated protein is derived from.

30 In an embodiment the polypeptides of the invention are at least 30% and less than 100% identical to a human protein, preferably over the whole length of the human

protein. Preferably the polypeptides are at least 40%, for example at least 50% identical to the human protein. More preferably the polypeptides are at least 60%, for example, at least 70% identical to the human protein. Most preferably the polypeptides are at least 85% identical to the human protein, for example, about
5 90% identical. Such proteins are capable of raising an immune response in humans that recognise the human protein.

For example, the UWGCG Package provides the BESTFIT program which can be used to calculate homology (for example used on its default settings) (Devereux *et al*
10 (1984) *Nucleic Acids Research* 12, p387-395). The PILEUP and BLAST algorithms can be used to calculate homology or line up sequences (typically on their default settings), for example as described in Altschul (1993) *J. Mol. Evol.* 36:290-300; Altschul *et al* (1990) *J. Mol. Biol.* 215:403-10.

15 Software for performing BLAST analyses is publicly available through the National Centre for Biotechnology Information (<http://www.ncbi.nlm.nih.gov/>). This algorithm involves first identifying high scoring sequence pair (HSPs) by identifying short words of length W in the query sequence that either match or satisfy some positive-valued threshold score T when aligned with a word of the same length in a database
20 sequence. T is referred to as the neighbourhood word score threshold (Altschul *et al*, 1990). These initial neighbourhood word hits act as seeds for initiating searches to find HSPs containing them. The word hits are extended in both directions along each sequence for as far as the cumulative alignment score can be increased. Extensions for the word hits in each direction are halted when: the cumulative
25 alignment score falls off by the quantity X from its maximum achieved value; the cumulative score goes to zero or below, due to the accumulation of one or more negative-scoring residue alignments; or the end of either sequence is reached. The BLAST algorithm parameters W , T and X determine the sensitivity and speed of the alignment. The BLAST program uses as defaults a word length (W) of 11, the
30 BLOSUM62 scoring matrix (see Henikoff and Henikoff (1992) *Proc. Natl. Acad. Sci.*

USA 89: 10915-10919) alignments (B) of 50, expectation (E) of 10, M=5, N=4, and a comparison of both strands, when the program is being used on polynucleotides.

The BLAST algorithm performs a statistical analysis of the similarity between two sequences; see e.g., Karlin and Altschul (1993) *Proc. Natl. Acad. Sci. USA* 90: 5873-5787. One measure of similarity provided by the BLAST algorithm is the smallest sum probability (P(N)), which provides an indication of the probability by which a match between two nucleotide or amino acid sequences would occur by chance. For example, a sequence is considered similar to another sequence if the smallest sum probability in comparison of the first sequence to the second sequence is less than about 1, preferably less than about 0.1, more preferably less than about 0.01, and most preferably less than about 0.001.

The successful design of a polypeptide according to the present invention can be verified for example by demonstrating that, when expressed in an appropriate host cell, the polypeptide adopts a conformation sufficiently similar to that of the self protein that antibodies are generated which are cross-reactive with the native self protein. This may be shown using immunological techniques, such as binding of monoclonal or polyclonal antibodies in ELISA, or by physicochemical techniques such as circular dichroism, or by crystallographic techniques such as X-ray crystallography or by computer modelling, or by numerous other approaches well known to those skilled in the art.

Further confirmation of a successful design can be obtained by administering the resulting polypeptide in a self-context in an appropriate vaccination regime, and observing that antibodies capable of binding the protein are induced. This binding may be assessed through use of ELISA techniques employing recombinant or purified native protein, or through bioassays examining the effect of the protein on a sensitive cell or tissue. A particularly favoured assessment is to observe a phenomenon causally related to activity of the protein in the intact host, and to determine whether the presence of antibodies induced by the methods of the

invention modulate that phenomenon. Thus a protein of the present invention will be able to raise antibodies to the native antigen in the species from which the native protein is derived.

- 5 The polypeptide of the invention may be further modified by mutation, for example substitution, insertion or deletion of amino-acids in order to add desirable properties (such as the addition of a sequence tag that facilitates purification or increase immunogenicity) or remove undesirable properties (such as an unwanted agonistic activity at a receptor) or trans-membrane domains. In particular the present
10 invention specifically contemplates fusion partners that ease purification such as poly histidine tags or GST expression partners that enhance expression.

In a preferred embodiment there is provided a human IL-13 having one or more of the following mutations or a conservative substitution thereof characteristic of mouse
15 IL-13. The following numbering refers to IL-13 expressed with its signal sequence in E.coli.

	R	→	K	at	position	30
	V	→	S	at	position	37
20	Y	→	F	at	position	63
	A	→	V	at	position	65
	E	→	D	at	position	68
	E	→	Y	at	position	80
	K	→	R	at	position	81
25	M	→	I	at	position	85
	G	→	H	at	position	87
	Q	→	H	at	position	113
	V	→	I	at	position	115
	D	→	K	at	position	117

More preferably the human IL-13 comprises at least two preferably at least 3,4,5,6 or more of the above mutations or a conservative substitution thereof. It is preferred that all twelve mutations are present.

5 A "conservative substitution" is one in which an amino acid is substituted for another amino acid that has similar properties, such that one skilled in the art of peptide chemistry would expect the secondary structure and hydrophobic nature of the polypeptide to be substantially unchanged.

10 For example, certain amino acids may be substituted for other amino acids in a protein structure without appreciable loss of interactive binding capacity with structures such as, for example, antigen-binding regions of antibodies or binding sites on substrate molecules. Since it is the interactive capacity and nature of a protein that defines that protein's biological functional activity, certain amino acid
15 sequence substitutions can be made in a protein sequence, and, of course, its underlying DNA coding sequence, and nevertheless obtain a protein with like properties. It is thus contemplated that various changes may be made in the peptide sequences of the disclosed compositions, or corresponding DNA sequences which encode said peptides without appreciable loss of their biological utility or activity.

20

In making such changes, the hydrophobic index of amino acids may be considered. The importance of the hydrophobic amino acid index in conferring interactive biologic function on a protein is generally understood in the art (Kyte and Doolittle, 1982, incorporated herein by reference). It is accepted that the relative hydrophobic
25 character of the amino acid contributes to the secondary structure of the resultant protein, which in turn defines the interaction of the protein with other molecules, for example, enzymes, substrates, receptors, DNA, antibodies, antigens, and the like. Each amino acid has been assigned a hydrophobic index on the basis of its hydrophobicity and charge characteristics (Kyte and Doolittle, 1982). These values
30 are: isoleucine (+4.5); valine (+4.2); leucine (+3.8); phenylalanine (+2.8); cysteine/cystine (+2.5); methionine (+1.9); alanine (+1.8); glycine (-0.4); threonine (-

0.7); serine (-0.8); tryptophan (-0.9); tyrosine (-1.3); proline (-1.6); histidine (-3.2); glutamate (-3.5); glutamine (-3.5); aspartate (-3.5); asparagine (-3.5); lysine (-3.9); and arginine (-4.5).

5 It is known in the art that certain amino acids may be substituted by other amino acids having a similar hydrophobic index or score and still result in a protein with similar biological activity, i.e. still obtain a biological functionally equivalent protein. In making such changes, the substitution of amino acids whose hydrophobic indices are within ± 2 is preferred, those within ± 1 are particularly preferred, and those within
10 ± 0.5 are even more particularly preferred. It is also understood in the art that the substitution of like amino acids can be made effectively on the basis of hydrophilicity. U. S. Patent 4,554,101 (specifically incorporated herein by reference in its entirety), states that the greatest local average hydrophilicity of a protein, as governed by the hydrophilicity of its adjacent amino acids, correlates with a biological property of the
15 protein.

As detailed in U. S. Patent 4,554,101, the following hydrophilicity values have been assigned to amino acid residues: arginine (+3.0); lysine (+3.0); aspartate (+3.0 \pm 1); glutamate (+3.0 \pm 1); serine (+0.3); asparagine (+0.2); glutamine (+0.2); glycine (0);
20 threonine (-0.4); proline (-0.5 \pm 1); alanine (-0.5); histidine (-0.5); cysteine (-1.0); methionine (-1.3); valine (-1.5); leucine (-1.8); isoleucine (-1.8); tyrosine (-2.3); phenylalanine (-2.5); tryptophan (-3.4). It is understood that an amino acid can be substituted for another having a similar hydrophilicity value and still obtain a biologically equivalent, and in particular, an immunologically equivalent protein. In
25 such changes, the substitution of amino acids whose hydrophilicity values are within ± 2 is preferred, those within ± 1 are particularly preferred, and those within ± 0.5 are even more particularly preferred.

As outlined above, amino acid substitutions are generally therefore based on the
30 relative similarity of the amino acid side-chain substituents, for example, their

hydrophobicity, hydrophilicity, charge, size, and the like. Exemplary substitutions that take various of the foregoing characteristics into consideration are well known to those of skill in the art and include: arginine and lysine; glutamate and aspartate; serine and threonine; glutamine and asparagine; and valine, leucine and isoleucine.

5 These are preferred conservative substitutions.

Amino acid substitutions may further be made on the basis of similarity in polarity, charge, solubility, hydrophobicity, hydrophilicity and/or the amphipathic nature of the residues. For example, negatively charged amino acids include aspartic acid and
10 glutamic acid; positively charged amino acids include lysine and arginine; and amino acids with uncharged polar head groups having similar hydrophilicity values include leucine, isoleucine and valine; glycine and alanine; asparagine and glutamine; and serine, threonine, phenylalanine and tyrosine. Other groups of amino acids that may represent conservative changes include: (1) ala, pro, gly, glu, asp, gln, asn, ser, thr;
15 (2) cys, ser, tyr, thr; (3) val, ile, leu, met, ala, phe; (4) lys, arg, his; and (5) phe, tyr, trp, his.

In a preferred embodiment, the mutated IL-13 of the present invention comprises one or more of the following sequences or a variant thereof comprising a
20 conservative substitution:

L K E L I E E L S N; (SEQ ID No 1)

F C V A L D S L; (SEQ ID No 2)

25

A I Y R T Q R I L H G; (SEQ ID No 3)

K I E V A H F I T K L L; (SEQ ID No 4)

30 The polypeptide of the invention is encoded by polynucleotides of the invention. A person skilled in the art will readily be able to determine the sequence of the

polynucleotide which encodes the polypeptide by applying the genetic code. Once the required nucleic acid sequence has been determined, the polynucleotide with the desired sequence can be produced as described in the examples. A skilled person will readily be able to adapt any parameters necessary, such as primers and
5 PCR conditions. It will also be understood by a person skilled in the art that, due to the degeneracy of the genetic code, there is potentially more than one polynucleotide which encodes a polypeptide of the invention.

The polynucleotide of the invention is typically RNA, for example mRNA, or DNA, for
10 example genomic DNA, cDNA or synthetic DNA. Preferably the polynucleotide is DNA. Particularly preferably it is cDNA.

The present invention further provides an expression vector, which is a nucleic acid construct, comprising the polynucleotide of the invention. Additionally, the nucleic
15 acid construct will comprise appropriate initiators, promoters, enhancers and other elements, such as for example, polyadenylation signals, which may be necessary, and which are positioned in the correct orientation, in order to allow for protein expression within a mammalian cell.

20 The promoter may be a eukaryotic promoter for example a CD68 promoter, Gal1, Gal10, or NMT1 promoter, a prokaryotic promoter for example Tac, Trc, or Lac, or a viral promoter, for example the cytomegalovirus promoter, the SV40 promoter, the polyhedrin promoter, the P10 promoter, or the respiratory syncytial virus LTR promoter. Preferably the promoter is a viral promoter. Particularly preferred is when
25 the promoter is the cytomegalovirus immediate early promoter, optionally comprising exon 1 from the HCMV IE gene.

The transcriptional regulatory elements may comprise enhancers, for example the hepatitis B surface antigen 3'untranslated region, the CMV enhancer; introns, for
30 example the CD68 intron, or the CMV intron A, or regulatory regions, for example the CMV 5' untranslated region.

The polynucleotide is preferably operably linked to the promoter on the nucleic acid construct such that when the construct is inserted into a mammalian cell, the polynucleotide is expressed to produce an encoded polypeptide.

The nucleic acid construct backbone may be RNA or DNA, for example plasmid DNA, viral DNA, bacterial DNA, bacterial artificial chromosome DNA, yeast artificial chromosome DNA, synthetic DNA. It is also possible for the nucleic acid construct to be artificial nucleic acid, for example phosphorothioate RNA or DNA. Preferably the construct is DNA. Particularly preferred is when it is plasmid DNA.

10 The present invention further provides a host cell comprising an expression vector of the invention. Such cells include transient, or preferably stable higher eukaryotic cell lines, such as mammalian cells or insect cells, using for example a baculovirus expression system, lower eukaryotic cells, such as yeast or prokaryotic cells such as bacterial cells. Particular examples of cells which may be modified by insertion of
15 vectors encoding for a polypeptide according to the invention include mammalian HEK293T, CHO, HeLa, NS0 and COS cells. Preferably the cell line selected will be one which is not only stable, but also allows for mature glycosylation of a polypeptide. Expression may be achieved in transformed oocytes. A polypeptide of the invention may be expressed in cells of a transgenic non-human animal,
20 preferably a mouse or expressed into the milk of larger mammals, such as goats, sheep and cows. A transgenic non-human animal expressing a polypeptide of the invention is included within the scope of the invention. A polypeptide of the invention may also be expressed in *Xenopus laevis* oocytes.

25 The present invention also includes pharmaceutical or vaccine compositions, which comprise a therapeutically effective amount of nucleic acid construct or polypeptide of the invention, optionally in combination with a pharmaceutically acceptable carrier, preferably in combination with a pharmaceutically acceptable excipient such as phosphate buffered saline (PBS), saline, dextrose, water, glycerol, ethanol,
30 liposomes or combinations thereof. The vaccine composition may alternatively comprise a therapeutically effective amount of a nucleic acid construct of the

invention, formulated onto metal beads, preferably gold beads. The vaccine composition of the invention may also comprise an adjuvant, such as, for example, in an embodiment, imiquimod, tucaresol or alum.

5 Protein adjuvant formulations are preferred as these induce high titre antibody responses.

Preferably the adjuvant is administered at the same time as of the invention and in preferred embodiments are formulated together. Such adjuvant agents
10 contemplated by the invention include, but this list is by no means exhaustive and does not preclude other agents: synthetic imidazoquinolines such as imiquimod [S-26308, R-837], (Harrison, et al. 'Reduction of recurrent HSV disease using imiquimod alone or combined with a glycoprotein vaccine', *Vaccine* 19: 1820-1826, (2001)); and resiquimod [S-28463, R-848] (Vasilakos, et al. ' Adjuvant activities of
15 immune response modifier R-848: Comparison with CpG ODN', *Cellular immunology* 204: 64-74 (2000).), Schiff bases of carbonyls and amines that are constitutively expressed on antigen presenting cell and T-cell surfaces, such as tucaresol (Rhodes, J. et al. ' Therapeutic potentiation of the immune system by costimulatory Schiff-base-forming drugs', *Nature* 377: 71-75 (1995)), cytokine, chemokine and co-
20 stimulatory molecules, Th1 inducers such as interferon gamma, IL-2, IL-12, IL-15 and IL-18, Th2 inducers such as IL-4, IL-5, IL-6, IL-10 and IL-13 and other chemokine and co-stimulatory genes such as MCP-1, MIP-1 alpha, MIP-1 beta, RANTES, TCA-3, CD80, CD86 and CD40L, other immunostimulatory targeting ligands such as CTLA-4 and L-selectin, apoptosis stimulating proteins and peptides
25 such as Fas, (49), synthetic lipid based adjuvants, such as vaxfectin, (Reyes et al., 'Vaxfectin enhances antigen specific antibody titres and maintains Th1 type immune responses to plasmid DNA immunization', *Vaccine* 19: 3778-3786) squalene, alpha-tocopherol, polysorbate 80, DOPC and cholesterol, endotoxin, [LPS], Beutler, B., 'Endotoxin, 'Toll-like receptor 4, and the afferent limb of innate immunity', *Current
30 Opinion in Microbiology* 3: 23-30 (2000)) ; CpG oligo- and di-nucleotides, Sato, Y. et al., 'Immunostimulatory DNA sequences necessary for effective intradermal gene

immunization', *Science* 273 (5273): 352-354 (1996). Hemmi, H. et al., 'A Toll-like receptor recognizes bacterial DNA', *Nature* 408: 740-745, (2000) and other potential ligands that trigger Toll receptors to produce Th1-inducing cytokines, such as synthetic Mycobacterial lipoproteins, Mycobacterial protein p19, peptidoglycan,
5 teichoic acid and lipid A.

Certain preferred adjuvants for eliciting a predominantly Th1-type response include, for example, a Lipid A derivative such as monophosphoryl lipid A, or preferably 3-de-O-acylated monophosphoryl lipid A. MPL[®] adjuvants are available from Corixa
10 Corporation (Seattle, WA; see, for example, US Patent Nos. 4,436,727; 4,877,611; 4,866,034 and 4,912,094). CpG-containing oligonucleotides (in which the CpG dinucleotide is unmethylated) also induce a predominantly Th1 response. Such oligonucleotides are well known and are described, for example, in WO 96/02555, WO 99/33488 and U.S. Patent Nos. 6,008,200 and 5,856,462. Immunostimulatory
15 DNA sequences are also described, for example, by Sato et al., *Science* 273:352, 1996. Another preferred adjuvant comprises a saponin, such as Quil A, or derivatives thereof, including QS21 and QS7 (Aquila Biopharmaceuticals Inc., Framingham, MA); Escin; Digitonin; or *Gypsophila* or *Chenopodium quinoa* saponins.

20
The present invention also provides methods of treating or preventing IL-13 mediated disease, any symptoms or diseases associated therewith, comprising administering an effective amount of a protein, a polynucleotide, a vector or a pharmaceutical composition according to the invention. Administration of a
25 pharmaceutical composition may take the form of one or more individual doses, for example in a "prime-boost" therapeutic vaccination regime. In certain cases the "prime" vaccination may be via particle mediated DNA delivery of a polynucleotide according to the present invention, preferably incorporated into a plasmid-derived vector and the "boost" by administration of a recombinant viral vector comprising the
30 same polynucleotide sequence, or boosting with the protein in adjuvant. Conversely the priming may be with the viral vector or with a protein formulation typically a

protein formulated in adjuvant and the boost with a DNA vaccine of the present invention.

For the treatment of self-antigen, for example IL-13, mediated disease it is preferred
5 that the adjuvant is a preferable inducer of a TH-1 response. In particular, the
adjuvant comprises an immunostimulatory CpG oligonucleotide, such as disclosed in
(WO96102555). Typical immunostimulatory oligonucleotides will be between 8-100
bases in length and comprises the general formula $X_1 \text{ CpGX}_2$ where X_1 and X_2 are
nucleotide bases, and the C and G are unmethylated.

10

The preferred oligonucleotides for use in adjuvants or vaccines of the present
invention preferably contain two or more dinucleotide CpG motifs preferably
separated by at least three, more preferably at least six or more nucleotides. The
oligonucleotides of the present invention are typically deoxynucleotides. In a
15 preferred embodiment the internucleotide in the oligonucleotide is
phosphorodithioate, or more preferably a phosphorothioate bond, although
phosphodiester and other internucleotide bonds are within the scope of the invention
including oligonucleotides with mixed internucleotide linkages. e.g. mixed
phosphorothioate/phosphodiesters. Other internucleotide bonds which stabilise the
20 oligonucleotide may be used. Methods for producing phosphorothioate
oligonucleotides or phosphorodithioate are described in US5,666,153, US5,278,302
and WO95/26204.

Examples of preferred oligonucleotides have the following sequences. The
25 sequences preferably contain phosphorothioate modified internucleotide linkages.

OLIGO 1: TCC ATG ACG TTC CTG ACG TT (CpG 1826) (SEQ ID NO 5)

OLIGO 2: TCT CCC AGC GTG CGC CAT (CpG 1758) (SEQ ID NO 6)

OLIGO 3: ACC GAT GAC GTC GCC GGT GAC GGC ACC ACG (SEQ ID NO 7)

30 OLIGO 4: TCG TCG TTT TGT CGT TTT GTC GTT (CpG 2006) (SEQ ID NO 8)

OLIGO 5: TCC ATG ACG TTC CTG ATG CT (CpG 1668) (SEQ ID NO 9)

Alternative CpG oligonucleotides may comprise the preferred sequences above in that they have inconsequential deletions or additions thereto.

The CpG oligonucleotides utilised in the present invention may be synthesized by any method known in the art (eg EP 468520). Conveniently, such oligonucleotides
5 may be synthesized utilising an automated synthesizer. An adjuvant formulation containing CpG oligonucleotide can be purchased from Qiagen under the trade name "ImmunEasy".

The compositions of the present invention may be used for both prophylaxis and
10 therapy. The present invention provides a polypeptide or a polynucleotide according to the invention for use in medicine. The invention further provides the use of a polypeptide or a polynucleotide of the invention in the manufacture of a medicament for the treatment of allergies, respiratory ailments such as asthma and COPD, helminth-infection related disorders, fibrosis or cirrhosis of the liver.

15

The present invention also provides a method of vaccinating which comprises administering an effective amount of a vaccine composition of the invention to a patient and provoking an immune response to the vaccine composition.

20 The present invention also provides vaccine compositions as described herein for use in vaccination of a mammal against IL-13 mediated disorders such as allergies, respiratory ailments, helminth-infection related disorders, fibrosis and cirrhosis of the liver. Respiratory ailments include, for example, asthma, such as allergic asthma, and chronic obstructive pulmonary disease (COPD). Specifically, a vaccine
25 composition capable of directing a neutralising response to IL-13 would therefore constitute a useful therapeutic for the treatment of asthma, particularly allergic asthma, in humans. It would also have application in the treatment of certain helminth infection-related disorders (Brombacher, 2000 *Bioessays* 22:646-656) and diseases where IL-13 production is implicated in fibrosis (Chiaramonte et al, 1999, *J*
30 *Clin Inv* 104:777-785), such as chronic obstructive pulmonary disease (COPD) and cirrhosis of the liver.

The vaccine compositions of the invention may be administered in a variety of manners for example via the mucosal, such as oral and nasal; pulmonary, intramuscular, subcutaneous or intradermal routes. Where the antigen is to be administered as a protein based vaccine, the vaccine will typically be formulated with
5 an adjuvant and may be lyophilised and resuspended in water for injection prior to use. Such compositions may be administered to an individual as an injectable composition, for example as a sterile aqueous dispersion, preferably isotonic. Typically such compositions will be administered intra muscularly, but other routes of administration are possible.

10

One technique for intradermally administration involves particle bombardment (which is also known as 'gene gun' technology and is described in US Patent No. 5371015). Proteins may be formulated with sugars to form small particles or DNA encoding the antigen may be coated on to inert particles (such as gold beads) and are accelerated
15 at speeds sufficient to enable them to penetrate a surface of a recipient (e.g. skin), for example by means of discharge under high pressure from a projecting device. (Particles coated with nucleic acid vaccine constructs of the invention and protein sugar particles are within the scope of the present invention, as are devices loaded with such particles.) Other methods of administering the nucleic acid constructs or
20 compositions containing said constructs directly to a recipient include ultrasound, electrical stimulation, electroporation and microseeding which is described in US-5,697,901.

A nucleic acid construct of the present invention may also be administered by means
25 of specialised delivery vectors useful in gene therapy. Gene therapy approaches are discussed for example by Verme *et al*, Nature 1997, 389:239-242. Both viral and non-viral systems can be used. Viral based systems include retroviral, lentiviral, adenoviral, adeno-associated viral, herpes viral and vaccinia-viral based systems. Non-viral based systems include direct administration of nucleic acids and liposome-
30 based systems. For example, the vectors may be encapsulated by liposomes or within polylactide co-glycolide (PLG) particles.

A nucleic acid construct of the present invention may also be administered by means of transformed host cells. Such cells include cells harvested from a subject. The nucleic acid vaccine construct can be introduced into such cells *in vitro* and the transformed cells can later be returned to the subject. The nucleic acid construct of the invention may integrate into nucleic acid already present in a cell by homologous recombination events. A transformed cell may, if desired, be grown up *in vitro* and one or more of the resultant cells may be used in the present invention. Cells can be provided at an appropriate site in a patient by known surgical or microsurgical techniques (e.g. grafting, micro-injection, etc.). Suitable cells include dendritic cells.

10

The amount of vaccine composition which is delivered will vary significantly, depending upon the species and weight of mammal being immunised, the nature of the disease state being treated/protected against, the vaccination protocol adopted (i.e. single administration versus repeated doses), the route of administration and the potency and dose of the adjuvant compound chosen. Based upon these variables, a medical or veterinary practitioner will readily be able to determine the appropriate dosage level but it may be, for example, when the vaccine is a nucleic acid that the dose will be 0.5-5 μ g/kg of the nucleic acid constructs or composition containing them. In particular, the dose will vary depending on the route of administration. For example, when using intradermal administration on gold beads, the total dosage will preferably be between 1 μ g – 10ng, particularly preferably, the total dosage will be between 10 μ g and 1ng. When the nucleic acid construct is administered directly, the total dosage is generally higher, for example between 50 μ g and 1 or more milligram. The above dosages are exemplary of the average case.

25

In a protein vaccine, the amount of protein in each vaccine dose is selected as an amount which induces an immunoprotective response without significant, adverse side effects in typical vaccinees. Such amount will vary depending upon which specific immunogen is employed and how it is presented. Generally, it is expected that each dose will comprise 1-1000 μ g of protein, preferably 1-500 μ g, preferably 1-

30

100µg, most preferably 1 to 50µg. An optimal amount for a particular vaccine can be ascertained by standard studies involving observation of appropriate immune responses in vaccinated subjects. Following an initial vaccination, subjects may receive one or several booster immunisation adequately spaced. Such a vaccine
5 formulation may be either a priming or boosting vaccination regime; be administered systemically, for example *via* the transdermal, subcutaneous or intramuscular routes or applied to a mucosal surface *via*, for example, *intra nasal* or oral routes.

There can, of course, be individual instances where higher or lower dosage ranges
10 are merited, and such are within the scope of this invention.

It is possible for the vaccine composition to be administered on a once off basis or to be administered repeatedly, for example, between 1 and 7 times, preferably between 1 and 4 times, at intervals between about 1 day and about 18 months, preferably
15 one month. This may be optionally followed by dosing at regular intervals of between 1 and 12 months for a period up to the remainder of the patient's life. In an embodiment the patient will receive the antigen in different forms in a prime boost regime. Thus for example an antigen will be first administered as a DNA based vaccine and then subsequently administered as a protein adjuvant base formulation.
20 Once again, however, this treatment regime will be significantly varied depending upon the size and species of animal concerned, the amount of nucleic acid vaccine and / or protein composition administered, the route of administration, the potency and dose of any adjuvant compounds used and other factors which would be apparent to a skilled veterinary or medical practitioner.

25

The following example illustrates the theory of the invention in mice rather than in humans, so that the protein is murine with mutations characteristic of human protein, but the results can readily be extrapolated to treatment of humans where the protein will have B cell epitopes from Human with mutations characteristic of a mouse, or
30 other analogous protein.

Throughout the following examples of the invention, use is made of various widely known and practised techniques in molecular and cellular biology. Practical details of these may be found in a number of textbooks including Sambrook et al (1989, 2nd edition. Cold Spring Harbor Press: New York). Amino acid sequences or
5 designations may be given in either the one letter code, or the three letter code. The prefix 'h' is used to denote a protein or gene of human origin, 'm', murine origin and 'c', a chimaeric construct. 'r' is used to indicate a recombinant protein.

Examples

10

1. Design of a vaccine against murine IL-13

IL-13 belongs to the SCOP (Murzin et al, 1995, *J Mol Biol* 247:536-540) defined 4-helical cytokines fold family. Individual members of this fold superfamily are related
15 structurally, but are difficult to align at the sequence level. The 3D structure of IL-13 has not yet been determined, but structures have been generated for a number of other 4-helical cytokines. Protein multiple sequence alignments were generated for IL-13 orthologues, and also for a number of other cytokines exhibiting this fold where the structure of at least one member had been determined (IL-4, GM-CSF, IL-5 and
20 IL-2). Secondary structure predictions were performed for the IL-13 protein multiple sequence alignment using DSC (King and Sternberg, 1996, *Prot Sci* 5:2298-2310), SIMPA96 (Levin, 1997, *Prot Eng* 7:771-776) and Pred2ary (Chandonia and Karplus, 1995, *Prot Sci* 4:275-285). The individual cytokine protein multiple sequence alignments were aligned to each other, using both the sequence information and the
25 structural information (from the known crystal structures and from the secondary structure prediction).

Antigenic sites, specifically B-cell epitopes, were predicted for murine IL-13 using the Cameleon software (Oxford Molecular), and these were mapped onto the IL-4
30 structure (accession number 1RCB in the Brookhaven database) using the protein multiple sequence alignment to give an idea of where they might be located

structurally on IL-13. From this analysis, exposed regions which were potentially both antigenic and involved in receptor binding were selected.

From this model, a chimaeric IL-13 sequence was designed in which the sequence of the predicted antigenic loops was taken from murine IL-13, and the sequence of the predicted structural (predominantly helical) regions was taken from human IL-13. The purpose of this design was to identify target epitopes from murine IL-13 against which neutralising antibodies might be raised, and to present them on a framework which was structurally similar to the native protein, but yet contained sufficient sequence variation to the native (murine) protein to ensure that one or more CD4 T helper epitopes would be present. The nucleic acid and protein sequences selected for this example of a chimaeric IL-13 vaccine are shown in Figure 1 (SEQ ID NO 19 and 20). The underlined sequences correspond to sequences found in the human orthologue. Twelve amino acids were substituted to achieve the sequence in figure 1. It should be understood that the degeneracy of the genetic code allows many possible nucleic acid sequences to encode identical proteins. Furthermore, it will be appreciated that there are other possible chimaeric IL-13 vaccine designs within the scope of the invention, that have other orthologous mutations in non-exposed areas.

1.2 Preparation of chimaeric IL-13

Chimaeric IL-13 (cIL-13) DNA sequence was synthesised from a series of partially overlapping DNA oligonucleotides, with the sequences cIL-13-1 to cIL-13-6 shown in Table 1. These oligos were annealed, and cIL-13 DNA generated by a PCR with the cycle specification of 94⁰C for 1 minute followed by 25 cycles of 94⁰C for 30 seconds, 55⁰C for 1 minute and 72⁰C for 2 minutes. Followed by 72⁰C for 7 minutes and cooling to 4⁰C when finished. The reaction product comprised a band of the expected size, 361 base pairs, which was subcloned into the T/A cloning vector pCR2.1 (Invitrogen, Groningen, Netherlands) to generate pCR2.1-cIL-13. A BamH1 and Xho1 cIL-13 digested fragment from pCR2.1-cIL-13 was then subcloned into the BamH1 and Xho1 sites in pGEX4T3 (Amersham Pharmacia, Amersham, Bucks, UK)

generating pGEX4T3-clL-13/1. On sequencing the pGEX4T3-clL-13/1 construct we discovered an extra 39 base pairs of DNA sequence (derived from the pCR2.1 vector) between the sequence for GST and clL-13. To correct this, we repeated the PCR for clL-13 using pGEX4T3-clL-13/1 and primers clL-13Fnew and clL-13R. The PCR product obtained was then cloned back into pGEX4T3 using BamH1 and Xho1 restriction sites, to generate the expression vector pGEX4T3-clL-13. The sequence of this construct was verified by dideoxy terminator sequencing. This vector encodes a genetic fusion protein consisting of glutathione-S-transferase and clL-13 (GST-clL-13). The two moieties of the protein are linked by a short spacer which contains the recognition site for thrombin. The fusion protein may be readily purified by glutathione sepharose affinity chromatography, and then used directly, or a preparation of free clL-13 produced by cleavage with thrombin.

Table 1. Oligonucleotides used to construct chimaeric IL-13.

15

Oligo	Sequence (5'-3')
clL-13-1R (SEQ ID NO 10)	TGTGATGTTGACCAGCTCCTCAATGAGCTCCCTAAGGG TCAGAGGGAGAGACACAGATCTTGGCACCGGCCC
clL-13-2F (SEQ ID NO 11)	AGGAGCTGGTCAACATCACACAAGACCAGACTCCCCT GTGCAACGGCAGCATGGTATGGAGTGTGGACCTGGC
clL-13-3R (SEQ ID NO 12)	GCAATTGGAGATGTTGGTCAGGGATTCCAGGGCTGCA CAGTACCCGCCAGCGGCCAGGTCCACACTCCATAC
clL-13-4F (SEQ ID NO 13)	TGACCAACATCTCCAATTGCAATGCCATCGAGAAGACC CAGAGGATGCTGGGCGGACTCTGTAACCGCAAGGC
clL-13-5R (SEQ ID NO 14)	AACTGGGCCACCTCGATTTTGGTATCGGGGAGGCTG GAGACCGTAGTGGGGCCTTGCGGTTACAGAGTCC
clL-13-6F (SEQ ID NO 15)	AAATCGAGGTGGCCAGTTTGTAAAGGACCTGCTCAG CTACACAAAGCAACTGTTTCGCCACGGCCCCTTC
clL-13F (SEQ ID NO 16)	CGCGGATTCGGGCCGGTGCCAAGATCTG
clL-13R	CTCCGCTCGAGTCGACTTAGAAGGGGCGGTGGCGAAA

(SEQ ID NO 17)	
cIL-13Fnew (SEQ ID NO 18)	CGCGGATCCGGGCCGGTGCCAAGATCTG

The pGEX4T3-cIL-13 expression vector was transformed into E.coli BLR strain (Novagen, supplied by Cambridge Bioscience, Cambridge, UK). Expression of GST-cIL-13 was induced by adding 0.5 mM IPTG to a culture in the logarithmic growth phase for 4hrs at 37⁰C. The bacteria were then harvested by centrifugation and GST-cIL-13 purified from them by a method previously described for purification of a similar GST-human IL-13 fusion protein (McKenzie et al, 1993, *Proc Natn Acad Sci* 90:3735-3739).

10 Characterisation of cIL-13 properties

Samples of purified GST-cIL-13 were analysed by SDS-PAGE electrophoresis. Figure 2 shows that the purified preparation contains a protein of the expected size for GST-cIL-13. The lower band represents a small quantity of GST, arising due to partial cleavage of the fusion protein during preparation.

To confirm that the purified protein was GST-cIL-13, samples were separated by SDS-PAGE, blotted onto PVDF membrane and then analysed for the presence of IL-13 and GST immunoreactivity by Western blotting. Since cIL-13 contains sequence arising from both human and murine IL-13, it was expected that it would be recognised by specific antisera directed at human IL-13 or mouse IL-13. Blots were blocked with 3% bovine serum albumin (BSA) in TBS (50mM trizma hydrochloride, 138mMsodium chloride, 2.7mM potassium chloride, pH8.0) containing 0.05% Tween-20 (TBST) overnight at 4⁰C, incubated with primary antibody for 1 hour at room temperature (RT) with shaking then washed 4 times with TBST. Secondary antibody was added for 1 hour at RT with shaking, prior to washing 4 times and developing with SuperSignal Chemiluminescent Reagent (Pierce, Rockford, Illinois, USA).

Figure 3 (legend below) illustrates the results of this analysis, which indicate that the purified protein is recognised by antibodies to human IL-13, mouse IL-13 and GST, so confirming the expected structure.

Lane	Sample	Primary Antibody
1	GST-clL-13	Anti-mIL-13
2	rhIL-13	Anti-mIL-13
3	rmIL-13	Anti-mIL-13
4	Markers	-
5	GST-clL-13	Anti-hIL-13
6	rhIL-13	Anti-hIL-13
7	rmIL-13	Anti-hIL-13
8	Markers	-
9	GST-clL-13	Anti-GST
10	rhIL-13	Anti-GST
11	rmIL-13	Anti-GST
12	GST	Anti-GST

5

The primary antibodies used in this experiment were: anti-hIL-13, catalogue number AF-213-NA, R&D Systems, Abingdon, Oxford, UK, used at 1µg/ml; anti-mIL-13, catalogue number AF-413-NA, R&D Systems, used at 1µg/ml and anti-GST, catalogue number 27-4590D, Pharmacia, used at 1/200. The secondary antibodies used in this experiment were: HRP-conjugated anti-goat IgG, catalogue number A-5420, Sigma-Aldrich Company Ltd, Poole, Dorset, UK, used at 1/40,000.

The protein samples were GST-clL-13, prepared as described in Example 2, recombinant human IL-13 (rhIL-13), catalogue number CH1-013, Cambridge Bioscience, Cambridge, UK, recombinant mouse IL-13 (rmIL-13) catalogue number 15 413-ML-025, R&D Systems, and GST, prepared from E.coli transfected with empty

pGEX4T3 vector as described (Sambrook et al, 1989, 2nd edition. Cold Spring Harbor Press: New York).

1.3 Conformation of Chimaeric IL-13

5

To confirm that GST-cIL-13 adopts a similar conformation in solution to that of native IL-13, samples of GST-cIL-13 and cIL-13 (generated from GST-cIL-13 by thrombin cleavage) were analysed by ELISA. 96-well Maxisorp plates (Life Technologies Ltd, Paisley, UK) were coated with cIL-13, GST-cIL-13, mL-13, hIL-13 or gst in
10 carbonate-bicarbonate buffer, overnight at 4⁰C. Plates were then blocked with 3% BSA/TBST for 1 hour at RT, washed 3 times in TBST, incubated with primary antibody for 1 hour at RT then washed 3 times in TBST. Secondary antibody was added for 1 hour, washed 3 times in TBST, then developed with 0-phenylenediamine dihydrochloride peroxidase substrate (OPD, Sigma Aldrich) for 30 minutes. The
15 primary and secondary antibodies used in this experiment were as described above. As shown in Figure 4, GST-cIL-3 and cIL-13 were specifically recognised by antibodies to human IL-13 and mouse IL-13. These data confirm that the chimaerisation process has not grossly altered the protein confirmation.

20 1.4 Binding of Chimaeric IL-13 to receptors

ELISAs were set up to determine whether cIL-13 could bind to either of the known mouse IL-13 receptors (mIL-13R1 or mIL-13R2). 96-well Maxisorp plates were coated with anti-human IgG (catalogue number I-3382, Sigma Aldrich) in carbonate-
25 bicarbonate buffer overnight at 4⁰C. Plates were then blocked with 3% BSA/TBST for 1 hour at RT, washed 3 times in TBST, and incubated with mL-13R1-Fc or mL-13R2-Fc (catalogue numbers 491-IR-200 and 539-IR-100 respectively, R+D Systems) for 1 hour at RT. After washing, plates were incubated with dilutions of mL-13 or cIL-13 or GST-cIL-13 for 1 hour at RT, washed again and incubated with
30 biotinylated anti-mIL-13 (catalogue number BAF413, R+D Systems). Following further washing and incubation with streptavidin conjugated horse-radish peroxidase,

the plates were developed with 0-phenylenediamine dihydrochloride peroxidase substrate for 30 minutes. As shown in Figure 5, cIL-13 and GST-cIL-13 are both able to bind to either of the mL-13 receptors. Again, these data confirm that the chimaerisation process has not grossly altered the protein confirmation.

5

1.5 Bioactivity of Chimaeric IL-13

The bioactivity of GST-cIL-13 was assessed by the ability of this protein to phosphorylate STAT6 in the human lung fibroblast cell line A549. These cells
 10 express the human type-2 IL-4 receptor that is responsive to both IL-4 and IL-13. Stimulation of these cells with hIL-4, hIL-13 or mL-13 induces phosphorylation of the signalling protein STAT6. 5×10^5 A549 cells were plated into 60mm tissue culture dishes (Life Technologies) in RPMI (Life Technologies) and grown to 70% confluence. Cells were then incubated with between 2 and 150ng/ml cytokine or
 15 purified cIL-13 for 15 mins at 37°C. Because the presence of a GST fusion partner may alter the bioactivity of cytokines, the chimaeric IL-13 was assayed as both GST-cIL-13 fusion protein, and free cIL-13 liberated from the fusion by thrombin cleavage. By way of control, mL-13 and GST were also tested. Cell lysates were then prepared and analysed by Western blot for the presence of phospho-STAT6 using
 20 rabbit anti-phospho-STAT6 polyclonal antibody (NEB, Hitchin, Herts, UK. Catalogue number 9361S). Blots were blocked overnight in 5% BSA / TBST (BSA must be A-7906 from Sigma as primary antibody is phospho-specific, 0.1% Tween-20), primary antibody was added at 1/1000 for 1hour at RT then washed 3 times with TBST. Anti-rabbit HRP conjugated secondary antibody (A-4914, Sigma Aldrich) was added at
 25 1/5000 for 1 hour at RT then washed 4 times with TBST prior to developing with the HRP chemiluminescent substrate ECL Reagent (Amersham Pharmacia). The results of this experiment are shown in Figure 6.

Each lane was loaded with the following protein:

30

Lane	Lysates of A549 cells treated with...
------	---------------------------------------

1	50ng/ml rmlL-13 (R&D Systems)
2	10ng/ml rmlL-13 (R&D Systems)
3	2ng/ml rmlL-13 (R&D Systems)
4	50ng/ml cIL-13
5	10ng/ml cIL-13
6	2ng/ml cIL-13
7	150ng/ml GST-cIL-13
8	30ng/ml GST-cIL-13
9	6ng/ml GST-cIL-13
10	No treatment
11	1µg/ml GST
12	0.25µg/ml GST
13	Molecular weight markers

Recombinant protein reagents were as described in Figure 3.

Treatment of A549 cells with 50 or 10ng/ml (but not 2ng/ml) rmlL-13 induced the phosphorylation of STAT6, indicating bioactivity. Treatment of A549 cells with 50ng/ml (but not 10 or 2ng/ml) cIL-13 induced the phosphorylation of STAT6, indicating bioactivity. Similarly, 150ng/ml GST-cIL-13 (which is approximately equivalent in molar terms to 50ng/ml cIL-13) is bioactive, whereas 30 and 6ng/ml are not. CIL-13 is therefore an agonist at this receptor, but under these experimental conditions is approximately 5 fold less bioactive than mIL-13.

1.6 Immunisation with cIL-13

cIL-13 and GST-cIL-13 were then used as immunogens to induce the formation of auto-antibodies against mouse IL-13 in Balb/c mice. Female mice aged 6-8 weeks were given one subcutaneous (sc) injection of approximately 30µg protein in complete Freund's adjuvant (CFA) at the base of the tail. This was followed by three

booster immunisations at the same site, each consisting of approximately 10 μ g protein in incomplete Freund's adjuvant [IFA] for boosts. Each treatment group contained 5 animals, and they were immunised according to the protocol in Table 2.

5 Table 2

Group	Immunisation
A	Saline control in CFA/IFA s/c
B	30/10 μ g GST in CFA/IFA s/c
C	Non immunised naïve mice
D	30/10 μ g GST-hIL-13 in CFA/IFA s/c
E	30/10 μ g cIL-13 in CFA/IFA s/c
F	30/10 μ g GST-cIL-13 in CFA/IFA s/c

Day	Treatment
-12	Pre-bleed
0	Primary immunisation
14	1 st Boost Immunisation
27	Tail bleed
42	Tail bleed
49	2 nd Boost Immunisation
70	Tail bleed
97	Tail bleed
99	3 rd Boost Immunisation
113	Tail bleed
140	Tail bleed

Serum samples were obtained by venepuncture of the tail vein at the timepoints specified in Table 2. After clarification by centrifugation, the samples were assayed
 10 by ELISA for the presence of specific IgG responses to mouse IL-13, human IL-13 and GST. None of the animals in groups A-D possessed anti-mouse IL-13 antibodies

at any time point. All of the animals in groups B, D and F made a strong IgG response to GST (group E animals also made strong antibody responses to GST, because there was GST remaining in the cIL-13 sample used to immunise these mice). Anti-mouse IL-13 antibody responses were induced in five out of five animals in group F and four out of five animals in group E. Figure 7 (a and b) shows the serological analysis for one of these animals in group F and one of these animals from group E 7b (gst – cIL-13 immunised and cIL-13 immunised respectively). The results indicate that immunisation with GST-cIL-13 or cIL-13 was able to break tolerance to mL-13, generating mouse anti-mIL-13 antibodies.

10

Sera from two mice (F1d70 and F5d97) that had strong anti-mIL-13 IgG responses, were tested for the capacity to neutralise the bioactivity of rmlL-13 in the A549/phospho-STAT6 assay. 20ng/ml or 10ng/ml rmlL-13 (R&D Systems) were incubated with 1% sera in serum free RPMI tissue culture media for 15 minutes at room temperature prior to a 15 minute incubation at 37°C with A549 cells. Cell lysates were prepared and analysed by Western blot for the presence of phospho-STAT6 as previously described above. As a negative control, anti-hIL-13 serum was obtained from a Balb/c mouse immunised with GST-hIL-13 and shown by ELISA to have a strong anti-hIL-13 IgG response, but no anti-mIL-13 antibodies. As a positive control, normal mouse serum was spiked with a neutralising anti-mIL-13 antibody (R&D Systems, catalogue number AF-413-NA) to give a final concentration of 1 µg/ml.

The results of this experiment are shown in Figure 8, in which the following was tested:

Lane	Cytokine	Antibody
1	20 ng/ml rmlL-13	Normal mouse serum
2	10 ng/ml rmlL-13	Normal mouse serum
3	0 ng/ml rmlL-13	Normal mouse serum

25

4	20 ng/ml rmlL-13	Serum sample F1d70
5	10 ng/ml rmlL-13	Serum sample F1d70
6	0 ng/ml rmlL-13	Serum sample F1d70
7	20 ng/ml rmlL-13	Anti-hIL-13 mouse serum
8	10 ng/ml rmlL-13	Anti-hIL-13 mouse serum
9	0 ng/ml rmlL-13	Anti-hIL-13 mouse serum
10	Molecular weight markers	-
11	0 ng/ml rmlL-13	Normal mouse serum + anti-mIL-13
12	20 ng/ml rmlL-13	Serum sample F5d97
13	10 ng/ml rmlL-13	Serum sample F5d97
14	0 ng/ml rmlL-13	Serum sample F5d97
15	20 ng/ml rmlL-13	Normal mouse serum + anti-mIL-13
16	10 ng/ml rmlL-13	Normal mouse serum + anti-mIL-13

Immunisation with a chimaeric IL-13 immunogen of the invention induces the production of auto-antibodies against mouse IL-13, capable of neutralising the biological activity of the mouse IL-13 (lanes 4, 5, 12, 13), in a fashion comparable to
 5 exogenously added anti-murine IL-13 antibody (lanes 15, 16). This activity is not present in normal mouse serum (lanes 1,2), nor in serum from animals immunised with GST-hIL-13 (lanes 7, 8).

These data provide a basis for treating mammals with an IL-13 dependent pathology
 10 by vaccinating them with cIL-13, and so inducing an endogenous neutralising antibody activity.

1.7 Alternative Constructs

15 1.7.1 6 his tagged cIL-13 design.

GST-cIL-13 is bacterially produced protein is insoluble and requires solubilisation and refolding *in vitro*. Size exclusion chromatography indicates that the refolding

process generates several differentially folded forms, which suggest that a proportion of the immune response is being directed against forms that may be generating irrelevant antibodies that do not bind native mouse IL-13.

- 5 Therefore this candidate may not be generating the most potent neutralising anti-mouse IL-13 antibody responses possible.

For this reason 6 his-clL-13 has been cloned into a mammalian expression vector, mammalian expressed 6 his-clL-13 is soluble and does not require refolding *in vitro*.

10

1.7.2 Figure 12 (SEQ ID NO 23 and 24) shows a vaccine antigen where different analogous mutations are made. Protein sequence numbering according to a scheme where the glycine residue in the sequence "GPVPR" is residue 1. Single underlined sequences correspond to the predicted helical regions from the revised structural model. Double underlined bold residues indicate points at which mutations are incorporated into the mouse sequence:

- 11 mouse Leu changed to Val (rat)
 21 mouse Ser changed to Thr (non-orthologous)
 20 63 mouse Tyr changed to Phe (non-orthologous)
 71 mouse Gly changed to Ala (dog/pig/cow)
 100 mouse Ser changed to Thr (dog)
 104 mouse Gln changed to Asn (non-orthologous)
 108 mouse His changed to Arg (non-orthologous)

25

1.8 Application to human therapy

Figure 9 shows one possible vaccine antigen according to the invention directed at the production of anti-human IL-13 antibodies in humans. This will be useful for the treatment of diseases characterised by excessive or inappropriate IL-13, for example asthma. The sequence corresponding to mouse IL-13 are underlined. The

30

construct contains twelve amino-acid substitutes that are analogous to murine IL-13.

These are:

	R	→	K	at	position	30
	V	→	S	at	position	37
5	Y	→	F	at	position	63
	A	→	V	at	position	65
	E	→	D	at	position	68
	E	→	Y	at	position	80
	K	→	R	at	position	81
10	M	→	I	at	position	85
	G	→	H	at	position	87
	Q	→	H	at	position	113
	V	→	I	at	position	115
	D	→	K	at	position	117

15

Figure 13 (SEQ ID NO 25) shows one possible vaccine for human use based on Chimaeric IL-4. It is an Example of a chimearic human IL-4 vaccine protein.

Underlined amino-acid residues comprise the alpha-helical structural regions and are derived from mouse IL-4 with the inclusion of amino acid 21 into the first helix.

20 Plain symbols indicate amino-acid residues derived from human IL-4. Positions of the alpha-helical regions are taken from Zuegg, J et al (2001) Immunol and Cell Biol 79:332-339.

**Example 2: Immune response to gst-clL-13 is specific for mouse IL-13 and
25 does not cross react with mouse IL-4.**

As mouse IL-13 is structurally similar to mouse IL-4, sera from a GST-clL-13 immunised mouse (that had been shown to contain high titre anti-mouse IL-13 autoantibodies) was analysed for cross-reactivity to mouse IL-4 using an anti-mouse
30 IL-4 ELISA and an *in vitro* mL-4 neutralisation bioassay.

2.1 Anti-mouse IL-4 ELISA.

96-well Maxisorp plates were coated with anti-mouse IL-4 monoclonal antibody (Cat. No. MAB404, R+D Systems) in carbonate-bicarbonate buffer overnight at 4°C.

5 Plates were then blocked with 3% BSA/TBST for 1 hour at RT, washed 3 times in TBST, and incubated with mouse IL-4 (Cat. No. 404-ML-005, R+D Systems) for 1 hour at RT. After washing, plates were incubated with mouse sera for 1 hour at RT, washed again and incubated with HRP conjugated anti-mouse IgG polyclonal antibody (Cat. No. A-9309, SIGMA). Following further washing, the plates were

10 developed with 0-phenylenediamine dihydrochloride peroxidase substrate for 30 minutes.

The level of anti-mouse IL-4 antibodies in the serum was expressed as an endpoint titre. The endpoint titre is defined as that dilution of serum that is equivalent to twice

15 the ELISA background reading.

Mouse	Anti-mouse IL-4 antibody endpoint titre	Anti-mouse IL-13 antibody endpoint titre
C2 (serum sample taken at day 125, post 4 x GST-clL-13 vaccine doses)	1/900	1/80000

A very low level of mouse IL-4 cross-reactivity was detected in this serum sample. In contrast, a much higher anti-mouse IL-13 antibody endpoint titre was previously

20 determined in this serum sample, using an anti-mouse IL-13 antibody ELISA. The level of mouse IL-4 cross-reactivity determined by this ELISA, would not be expected to have mouse IL-4 neutralising effects *in vivo*. This serum sample was assessed for mouse IL-4 neutralisation capacity in an *in vitro* mouse IL-4 bioassay.

2.2 In vitro mouse IL-4 neutralisation bioassay.

Mouse IL-4 stimulates the proliferation of CTLL cells *in vitro*. An assay was therefore developed in these cells, to assess the mouse IL-4 neutralisation capacity of serum
5 from this GST-cIL-13 vaccinated mouse.

To measure the ability of mouse serum to neutralise the bioactivity of recombinant mouse IL-4 on mouse CTLL cells (Cat. No. 87031904, ECACC), 3ng/ml recombinant mouse IL-4 was incubated with various concentrations of sera for 1 hour at 37°C in a
10 96-well tissue culture plate (Invitrogen). Following this pre-incubation period, CTLL cells were added. The assay mixture, containing various serum dilutions, recombinant mouse IL-4 and CTLL cells, was incubated at 37°C for 70 hours in a humidified CO₂ incubator. MTT substrate (Cat. No. G4000, Promega) was added during the final 4 hours of incubation, after which the reaction was stopped with an
15 acid solution to solubilise the metabolised blue formazan product. The absorbance of the solution in each well was read in a 96-well plate reader at 570nm wavelength.

Note that this assay is only able to measure mouse IL-4 neutralisation capacity in serum dilutions greater than or equivalent to 1/100. Serum dilutions less than 1/100
20 induce non-specific proliferative effects in CTLL cells.

The capacity of the serum to neutralise mouse IL-4 bioactivity was expressed as, that dilution of serum required to neutralise the bioactivity of a defined amount of mouse IL-4 by 50% (= ND₅₀). The more dilute serum sample required, the more
25 potent the neutralisation capacity.

The highest concentration of mouse C2 serum tested was a 1/100 dilution. This did not neutralise the bioactivity of 3ng/ml mouse IL-4 by 50%, therefore the ND₅₀ is expressed as < 1/100 dilution.

Mouse	Mouse IL-4 neutralisation capacity (ND ₅₀)	Mouse IL-13 neutralisation capacity (ND ₅₀)
C2 (serum sample taken at day 125, post 4 x GST- cIL-13 vaccine doses)	<1/100	1/5300

No mouse IL-4 neutralisation capacity was detected in this serum sample at the dilutions of serum tested. In contrast (when assessed for mouse IL-13 neutralisation capacity), this serum sample potentially neutralised mouse IL-13 bioactivity.

These data demonstrate that although a very low level of mouse IL-4 cross-reactivity can be measured in the serum by an anti-mouse IL-4 antibody ELISA, there is no associated mouse IL-4 neutralisation capacity.

10

2.3 New mouse IL-13 neutralisation bioassay to assess the mouse IL-13 neutralisation capacity of mouse serum samples.

Previous GST-cIL-13 bioactivity and mouse IL-13 neutralisation capacity data were generated using a STAT-6 phosphorylation readout in A549 cells. This assay was cumbersome and not easily amenable for the generation of quantitative data. Mouse IL-13 stimulates the proliferation of TF-1 cells *in vitro*. An assay was therefore developed in these cells to assess the mouse IL-13 neutralisation capacity of serum from GST-cIL-13 vaccinated mice.

20

2.4 In vitro mouse IL-13 neutralisation bioassay.

To measure the ability of mouse serum to neutralise the bioactivity of recombinant mouse IL-13 on human TF-1 cells (obtained in-house), 5ng/ml recombinant mouse IL-13 was incubated with various concentrations of sera for 1 hour at 37°C in a 96-well tissue culture plate (Invitrogen). Following this pre-incubation period, TF-1 cells were added. The assay mixture, containing various serum dilutions, recombinant

25

mouse IL-13 and TF-1 cells, was incubated at 37°C for 70 hours in a humidified CO₂ incubator. MTT substrate (Cat. No. G4000, Promega) was added during the final 4 hours of incubation, after which the reaction was stopped with an acid solution to solubilise the metabolised blue formazan product. The absorbance of the solution in each well was read in a 96-well plate reader at 570nm wavelength.

Note that this assay is only able to measure mouse IL-13 neutralisation capacity in serum dilutions greater than or equivalent to 1/100. Serum dilutions less than 1/100 induce non-specific proliferative effects in TF-1 cells.

- 10 The capacity of the serum to neutralise mouse IL-13 bioactivity was expressed as, that dilution of serum required to neutralise the bioactivity of a defined amount of mouse IL-13 by 50% (= ND₅₀). The more dilute serum sample required, the more potent the neutralisation capacity.
- 15 The mouse IL-13 neutralisation capacity of serum from GST-clL-13 immunised mice was measured by the above method. Potent IL-13 neutralising responses were generated, as indicated below.

Mouse (Serum samples taken at day 125, post 4 x GST-clL-13 vaccine doses)	Mouse IL-13 neutralisation capacity (ND ₅₀)
C1	1/1250
C2	1/5230
C3	1/523
C4	1/417
C5	1/1670

2.5 Determination of the level of mouse IL-13 neutralisation required for efficacy in the 'ovalbumin challenge' mouse asthma model.

In order to benchmark the required potency of an IL-13 autovaccine for treatment of asthma, mice were treated with various doses of rabbit anti-mouse IL-13 polyclonal antibody (administered passively by intra-peritoneal injection) during ovalbumin challenge, in the 'ovalbumin challenge' mouse asthma model. Model parameters such as airway hyper-responsiveness (AHR), goblet cell metaplasia (GCM) and lung inflammatory cell content were measured at the end of this experiment. Efficacy in this model was correlated to the levels of mouse IL-13 neutralisation achieved in mouse serum. The mouse IL-13 neutralisation bioassay was used to determine the level of mouse IL-13 neutralisation in serum samples.

Treatment group (Dose of passively administered rabbit anti-mouse IL-13 antibody)	Mouse IL-13 neutralisation capacity (ND ₅₀)
Highest dose	1/4100
High dose	1/2670
Mid dose	1/476
Lowest dose	1/207

15

Treatment groups given the highest three doses of antibody all performed similarly. All of these three groups showed efficacy equivalent to (for AHR) or better than (for GCM) the gold standard treatment (dexamethasone, administered by the intraperitoneal route at 3 x 1.5mg/kg) used in this model. The 'lowest dose' of antibody administered, showed efficacy somewhere between that of dexamethasone and the 'no treatment' positive control groups.

Therefore the level of IL-13 neutralisation achieved in the 'mid dose' treatment group, represents the required potency threshold for an IL-13 autovaccine in this

animal model. The potency threshold is defined as the lowest level of IL-13 neutralisation in mouse serum, required to show 100% efficacy in the asthma model (= ED₁₀₀). 1x ED₁₀₀ is therefore equivalent to an ND₅₀ of 1/476.

5 Significance of defined potency threshold.

The level of IL-13 neutralisation required for efficacy in the 'ovalbumin challenge' mouse asthma model has been defined above. The levels of IL-13 neutralisation induced by GST-cIL-13 in mice C1-3 and C5, are in excess of the potency threshold
10 required for efficacy in the asthma model. These results are illustrated in figure 11.

Therefore the GST-cIL-13 vaccine would be expected to show efficacy in the mouse asthma model.

15 Example 3: Immunogenicity profile of GST-cIL-13 in combination with various adjuvants.

3.1 Immunisation protocol.

20 GST-cIL-13 was used as an immunogen to induce the formation of auto-antibodies against mouse IL-13 in Balb/c mice. Female mice aged 6-8 weeks were given one injection of approximately 100µg protein in adjuvant. This was followed by four booster immunisations each consisting of 50µg protein in adjuvant (See below for immunogen + adjuvant formulations). Each treatment group contained 5 animals,
25 immunised according to the protocol in the table below.

Serum samples were obtained by venepuncture of the tail vein at the timepoints specified. After clarification by centrifugation, the samples were assayed by ELISA for the presence of specific IgG responses to mouse IL-13.

Group	Immunisation
A	GST-clL-13 in AS03 i/m
B	GST-clL-13 in Alum i/p
C	GST-clL-13 in 'ImmunEasy' i/m
D	GST-clL-13 in CFA/IFA s/c
E	GST-clL-13 in PBS s/c
F	No immunisations

Day	Treatment
-7	Pre-bleed
0	Primary immunisation
21	1 st boost immunisation
35	Tail bleed
49	2 nd boost immunisation
63	Tail bleed
77	3 rd boost immunisation
92	Tail bleed
106	4 th boost immunisation
125	Tail bleed

3.2 Immunogen + adjuvant formulation.

5

Preparation of emulsion adjuvant AS03:

Tween 80 is dissolved in phosphate buffered saline (PBS) to give a 2% solution in the PBS. To provide 100 ml two-fold concentrate emulsion 5g of DL alpha
 10 tocopherol and 5ml of squalene are vortexed to mix thoroughly. 90ml of PBS/Tween solution is added and mixed thoroughly. The resulting emulsion is then passed through a syringe and finally microfluidised by using an M110S microfluidics machine. The resulting oil droplets have a size of approximately 180 nm.

Mix adjuvant 1:1 with protein solution, vortex briefly (10 seconds at middle speed) and incubate for 10 minutes at room temperature on an orbital shaker. Vortex briefly before injection and administer 100ul total suspension per mouse by the intramuscular route at 2 separate sites (ie. 2 x 50ul per mouse, one injection in each quadriceps muscle). Prepare fresh before each immunisation.

Alum

Supplied by SIGMA (Cat. No. A-1577). Prepare a 2mg/ml suspension of alum in PBS. Mix adjuvant 1:1 with protein solution, vortex briefly and incubate shaking gently for 10 minutes at room temperature. Vortex briefly before injection and administer 100ul total suspension per mouse i/p. Prepare fresh before each immunisation.

CpG - ImmunEasy

Supplied by Qiagen (Cat.No. 303101). Mix the stock pot of adjuvant by gentle vortexing, then mix adjuvant 1:1 with protein by gently pipetting up and down 5 times. Incubate at room temperature for 15 minutes. Gently pipette the mix up and down 5 times and administer 100ul suspension per mouse by the intramuscular route at 2 separate sites (ie. 2 x 50ul per mouse, one injection in each quadriceps muscle). Prepare fresh before each immunisation.

CFA/IFA

Supplied by SIGMA (Cat. Nos. F-5881, F-5506). Formulate 1:1 with pre-mixed CFA for primary or IFA for boosts. Whirlimix sample to ensure an even white suspension with the CFA/IFA. Store on ice for at least 30 mins prior to use and whirlimix thoroughly prior to dosing.

3.3 Anti-mouse IL-13 antibody responses.

Anti-mouse IL-13 antibody responses were monitored in the serum samples using an anti-mouse IL-13 antibody detection ELISA.

96-well Maxisorp plates were coated with anti-mouse IL-13 monoclonal antibody (Cat. No. MAB, R+D Systems) in carbonate-bicarbonate buffer overnight at 4°C. Plates were then blocked with 3% BSA/TBST for 1 hour at RT, washed 3 times in TBST, and incubated with mouse IL-13 (Cat. No. 413-ML-025, R+D Systems) for 1
 5 hour at RT. After washing, plates were incubated with mouse sera for 1 hour at RT, washed again and incubated with HRP conjugated anti-mouse IgG polyclonal antibody (SIGMA, Cat. No. A-9309). Following further washing the plates were developed with 0-phenylenediamine dihydrochloride peroxidase substrate for 30 minutes.

10

The level of anti-mouse IL-13 antibodies in the serum was expressed as an endpoint titre. The endpoint titre is defined as that dilution of serum that is equivalent to twice the ELISA background reading.

Mouse	Anti-mouse IL-13 antibody endpoint titre			
	AS03	Alum	CpG	CFA/IFA
1	1/875	1/7250	1/67500	1/6750
2	1/9250	1/800	1/80000	1/975
3	1/160	1/9000	1/54000	1/6000
4	1/9000	1/6500	1/62500	1/16000
5	1/3600	1/10000	1/77500	1/31000

15

Figure 10 illustrates the anti-mouse IL-13 antibody profiles in the various treatment groups at day 125, for serum samples diluted at 1/100.

20 All five mice immunised with GST-cIL-13 in combination with CpG adjuvant raised strong anti-mouse IL-13 auto-antibody responses. This is in contrast to the other adjuvants, where responses were less consistent throughout each group, some mice raising very weak responses indeed.

25 These results indicate that CpG adjuvant is much more effective at raising consistent high titre anti-mouse IL-13 auto-antibody responses compared to the other adjuvants tested.

These serum samples were analysed for IL-13 neutralising ability in an *in vitro* IL-13 neutralisation bioassay.

3.4 IL-13 neutralisation capacity.

5

To measure the ability of mouse serum to neutralise the bioactivity of recombinant mouse IL-13 on human TF-1 cells (ATCC Cat. No. CRL-2003), 5ng/ml recombinant mouse IL-13 was incubated with various concentrations of sera for 1 hour at 37°C in a 96-well tissue culture plate (Gibco BRL). Following this pre-incubation period, TF-1
10 cells were added. The assay mixture, containing various serum dilutions, recombinant mouse IL-13 and TF-1 cells, was incubated at 37°C for 70 hours in a humidified CO₂ incubator. MTT substrate (Cat. No. G4000, Promega) was added during the final 4 hours of incubation, after which the reaction was stopped with an acid solution to solubilise the metabolised blue formazan product. The absorbance
15 of the solution in each well was read in a 96-well plate reader at 570nm wavelength.

Note that this assay is only able to measure mouse IL-13 neutralisation capacity in serum dilutions greater than or equivalent to 1/100. Serum dilutions less than 1/100 induce non-specific proliferative effects in TF-1 cells.

20

The capacity of the serum to neutralise mouse IL-13 bioactivity was expressed as, that dilution of serum required to neutralise the bioactivity of 5ng/ml mouse IL-13 by 50% (= ND₅₀). The more dilute serum sample required, the more potent the neutralisation capacity.

25

The highest concentration of mouse D5 serum tested was a 1/100 dilution. This did not neutralise the bioactivity of 5ng/ml mouse IL-13 by 50%, therefore the ND₅₀ is expressed as < 1/100 dilution.

30

Mouse (Serum samples taken at day 125)	Mouse IL-13 neutralisation capacity (ND ₅₀)
C1	1/1250
C2	1/5230
C3	1/523
C4	1/417
C5	1/1670
D5	<1/100

Day 125 serum samples from all five mice immunised with GST-clL-13 in combination with CpG adjuvant, were able to potently neutralise the bioactivity of mouse IL-13 in an *in vitro* bioassay. In contrast, the day 125 serum sample from mouse D5 (immunised with GST-clL-13 in CFA/IFA) was unable to neutralise the bioactivity of mouse IL-13 at all dilutions tested.

These results indicate that CpG adjuvant is much more effective at raising neutralising anti-mouse IL-13 auto-antibody responses compared to the other adjuvants tested.

Claims

1. An isolated protein which is at least 30% but less than 100% identical to a human protein which polypeptide
5 (a) contains at least one mutation which is characteristic of an analogous non-human protein;
(b) is capable of raising antibodies in human
(c) is sufficiently structurally similar to the human protein that the antibodies bind to both the human protein and the polypeptide
10 and
wherein the protein is not an antibody.

2. A protein having B-cell epitopes from a mammalian self-antigen and a mutation that gives rise to a sequence of an analogous protein of a second
15 mammalian species such that the protein is able to raise in the species from which the B-cell epitopes derived, an immune response that recognises the natural protein from which the B-cell epitopes are derived.

3. A protein having B-cell epitopes of a self-protein which are grafted, by
20 substitution, into a frame work of an analogous protein from a second mammalian species such that the protein is able to raise in the species in which the B-cell epitopes are derived, an immune response that recognises the natural protein from which the B-cell epitopes are derived.

- 25 4. A protein as claimed in any of claims 1 to 3 comprising conserved surface region introduced into the non-surface exposed region, said mutation giving rise to a sequence of an analogous protein such that the protein is able to raise an immune response to the self protein in the species from which the self-protein is derived.

- 30 5. A protein as claimed in any one of claims 1 to 4 wherein the immune response is a neutralising antibody response.

6. A protein as claimed in any of claims 1 to 5 wherein the human protein, or B-cell epitope is derived from a cytokine.
- 5 7. A cytokine as claimed in claim 6, which is a 4-helical cytokine.
8. A cytokine as claimed in claim 7 which is IL-4 or IL-13.
9. A mutated human – IL-13 having one or more of the following substitutions or
10 a substitution involving a conservative substitution thereof:

	R	→	K	at	position	30
	V	→	S	at	position	37
	Y	→	F	at	position	63
15	A	→	V	at	position	65
	E	→	D	at	position	68
	E	→	Y	at	position	80
	K	→	R	at	position	81
	M	→	I	at	position	85
20	G	→	H	at	position	87
	Q	→	H	at	position	113
	V	→	I	at	position	115
	D	→	K	at	position	117

- 25 10. A mutated human IL-13 as claimed in claim 9 having a plurality of substitutions as set forth in claim 9.
11. A mutated human IL-13 as claimed in any of claim 9 or 10 having one or more of the following sequences

30

L K E L I E E L S N

F C V A L D S L

A I Y R T Q R I L H G

5

K I E V A H F I T K L L

or a variant of said sequence comprising one or more conservative substitutions.

- 10 12. A mutated human IL-13 as shown in figure 9.
13. A polynucleotide encoding a protein of claim 1 to 12.
14. A polynucleotide of claim 13 which is a DNA and is operably linked to a
15 promoter.
15. A vector comprising a polynucleotide of claim 13 or 14.
16. A host transformed with a polynucleotide of claim 13 or 14 or vector of claim
20 15.
17. A pharmaceutical composition comprising , a protein, polynucleotide, vector
as claimed in any of claims 1 to 15 with a pharmaceutically acceptable carrier or
excipient.
- 25
18. A pharmaceutical composition as claimed in claim 17 additionally comprising
an adjuvant.
19. A pharmaceutical composition as claimed in claim 18 comprising a protein as
30 set forth in any of claims 1 to 12 and an immunostimulatory oligonucleotide.

20. A pharmaceutical composition as claimed in claim 19 wherein the immunostimulatory oligonucleotide is selected from the group:

OLIGO 1(SEQ ID NO:1): TCC ATG ACG TTC CTG ACG TT (CpG 1826)

5 OLIGO 2 (SEQ ID NO:2): TCT CCC AGC GTG CGC CAT (CpG 1758)

OLIGO 3(SEQ ID NO:3): ACC GAT GAC GTC GCC GGT GAC GGC ACC ACG

OLIGO 4 (SEQ ID NO:4): TCG TCG TTT TGT CGT TTT GTC GTT (CpG 2006)

OLIGO 5 (SEQ ID NO:5): TCC ATG ACG TTC CTG ATG CT (CpG 1668)

10 21. A protein, polynucleotide, vector, host or composition as claimed in any of claims 1 to 20 for use in medicine.

22. Use of a protein as claimed in any of claim 1 to 12 in the manufacture of a medicament for the treatment of IL-13 mediated diseases.

15

23. Use as claimed in claim 22 for the treatment of asthma.

24. A method for the treatment of prophylaxis of IL-13 mediated disease comprising the administration of a safe and effective amount of composition
20 according to any of claims 17-20 to a patient in need thereof.

25. A method for the preparation of a protein according to any of claims 1 to 12 which method comprises:

- 25 1. identification of one or more regions of a self, typically human, protein against which an antibody response is desired.
2. identification of the amino-acid sequence of the self protein.
3. identification of the amino-acid sequence of an analogous protein construction by recombinant DNA techniques of a chimaeric molecule containing
30 at least one target region identified in step 1, whose amino-acid sequence is taken from the sequence identified in step 2, and

55

sufficient amino-acids from the sequence(s) identified in step 3 to enable the resulting protein to fold into a shape similar to that the self protein such that the mutated protein can raise an immune response that recognises the self protein.

5

10

15

20

25

30

Figure 1.

```

5      GGGCCGGTGCCAAGATCTGTGTCTCTCCCTCTGACCCTTAGGGAGCTCATTGAGGAGCTG
1      -----+-----+-----+-----+-----+-----+-----+
      G P V P R S V S L P L T L R E L I E E L                                60

10     GTCAACATCACACAAGACCAGACTCCCCTGTGCAACGGCAGCATGGTATGGAGTGTGGAC
61     -----+-----+-----+-----+-----+-----+-----+
      V N I T Q D Q T P L C N G S M V W S V D                                120

15     CTGGCCGCTGGCGGGTACTGTGCAGCCCTGGAATCCCTGACCAACATCTCCAATTGCAAT
121    -----+-----+-----+-----+-----+-----+-----+
      L A A G G Y C A A L E S L T N I S N C N                                180

20     GCCATCGAGAAGACCCAGAGGATGCTGGGCGGACTCTGTAACCGCAAGGCCCCCACTACG
181    -----+-----+-----+-----+-----+-----+-----+
      A I E K T Q R M L G G L C N R K A P T T                                240

25     GTCTCCAGCCTCCCCGATACCAAATCGAGGTGGCCAGTTTGTAAGGACCTGCTCAGC
241    -----+-----+-----+-----+-----+-----+-----+
      V S S L P D T K I E V A Q F V K D L L S                                300

30     TACACAAAGCAACTGTTTCGCCACGGCCCTTCTAA
301    -----+-----+-----+-----+-----+-----+-----+
      Y T K Q L F R H G P F *                                336
    
```

Figure 2.

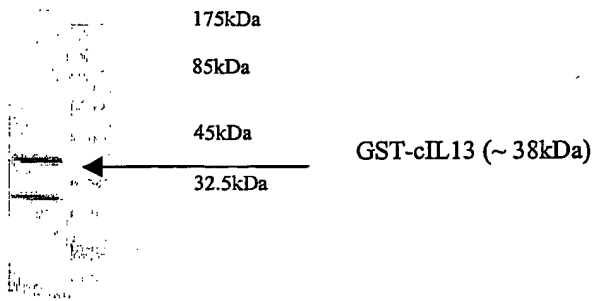


Figure 3

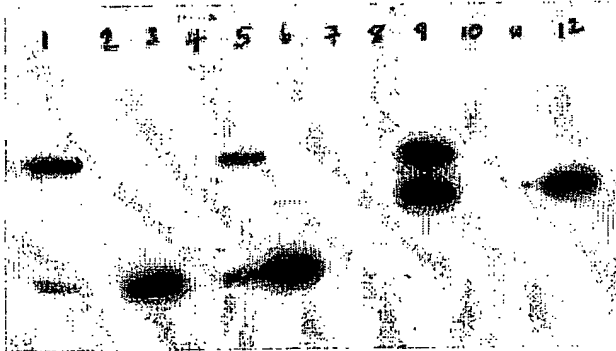


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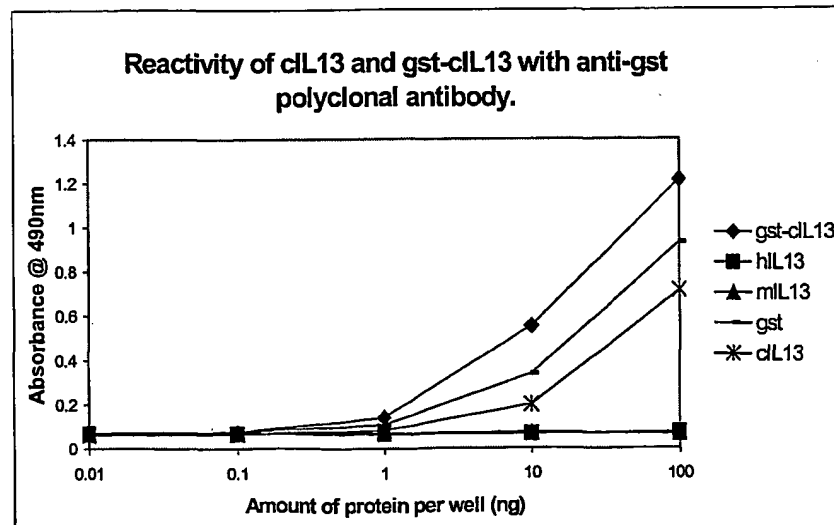
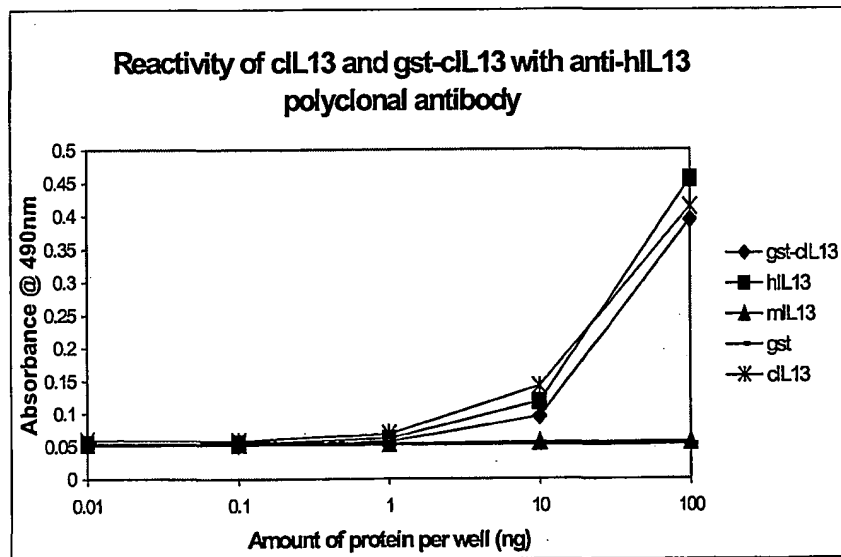
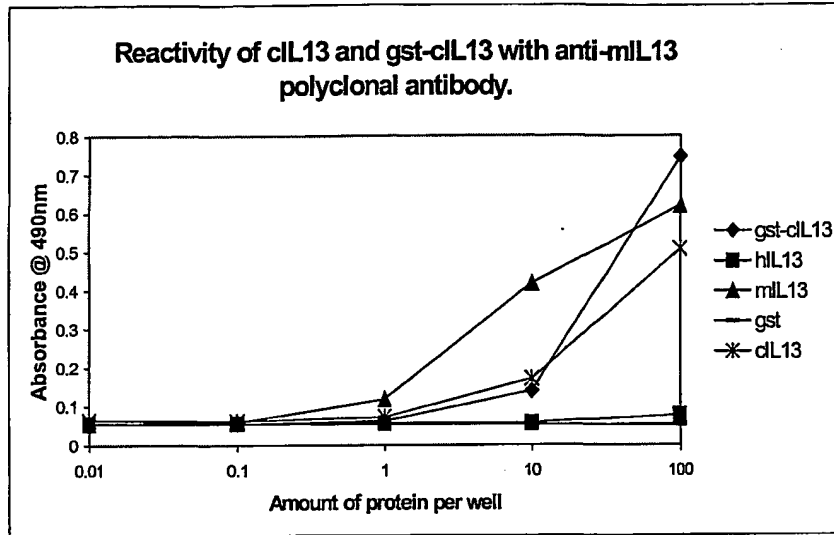


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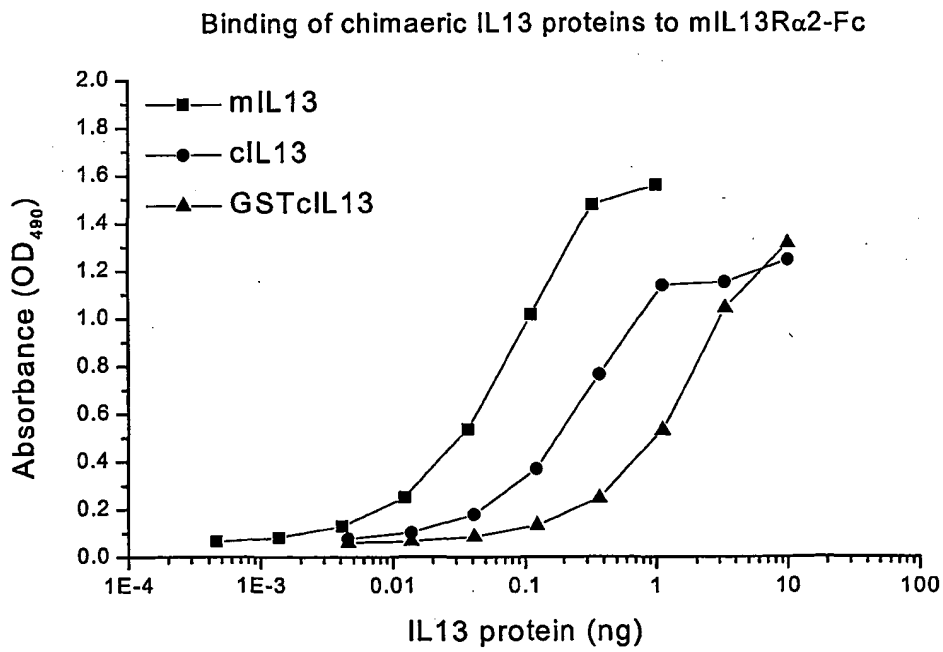
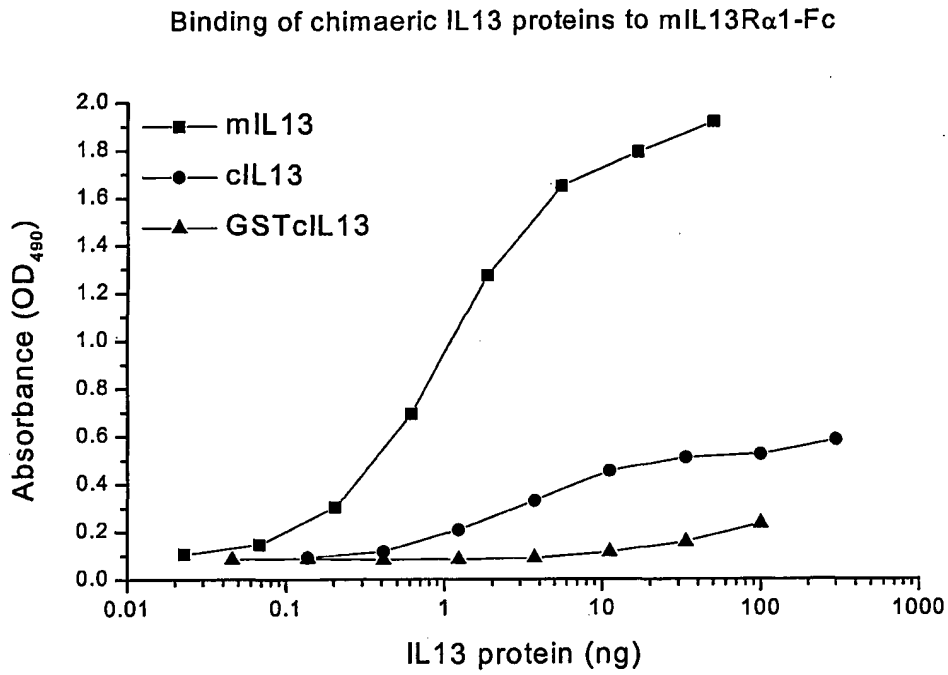
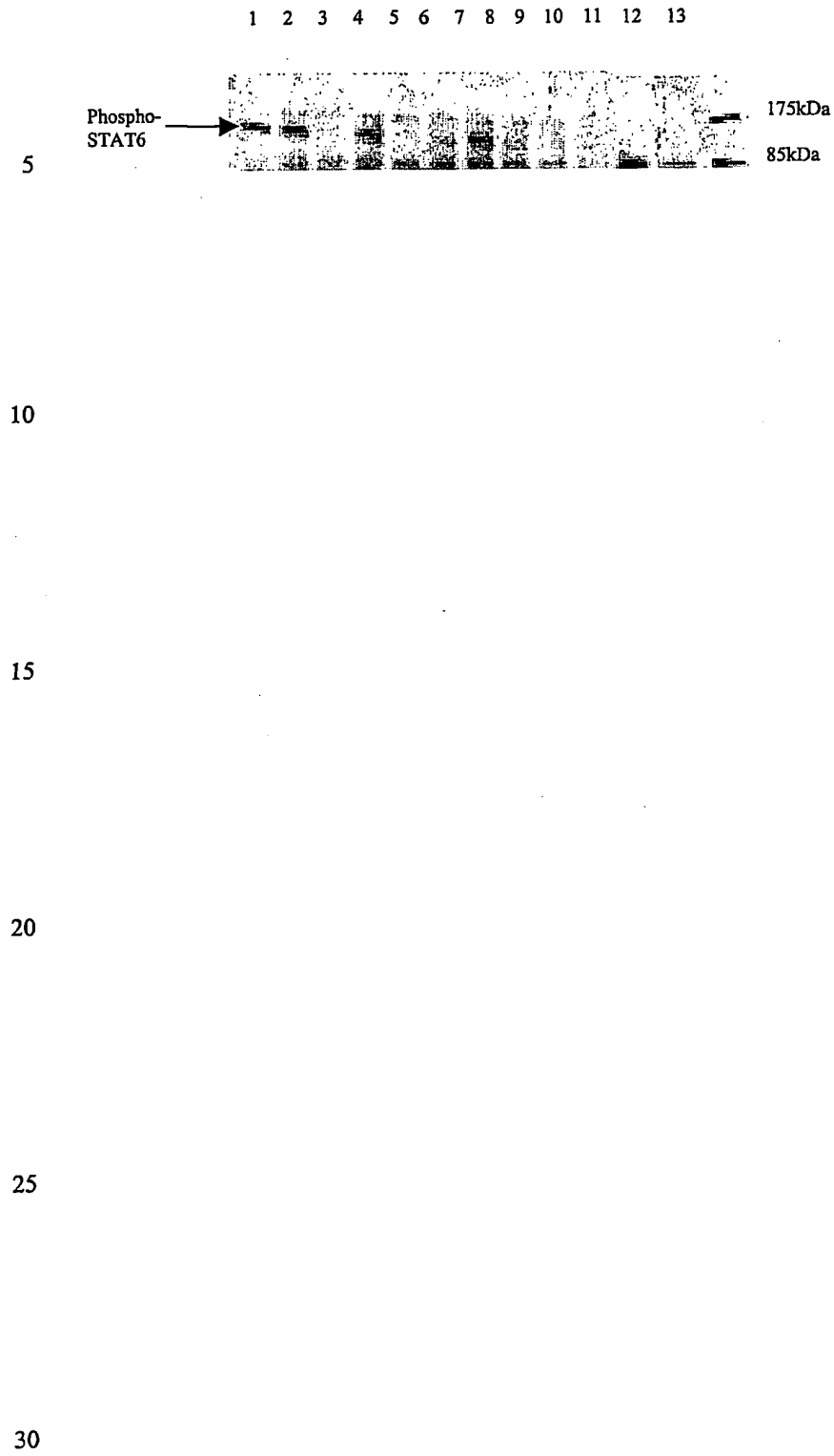


Figure 6



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Figure 7a

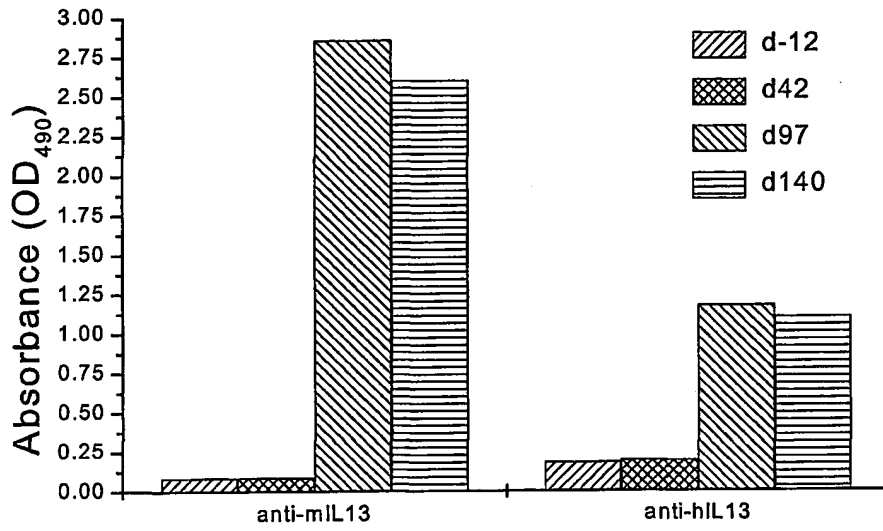


Figure 7b

5

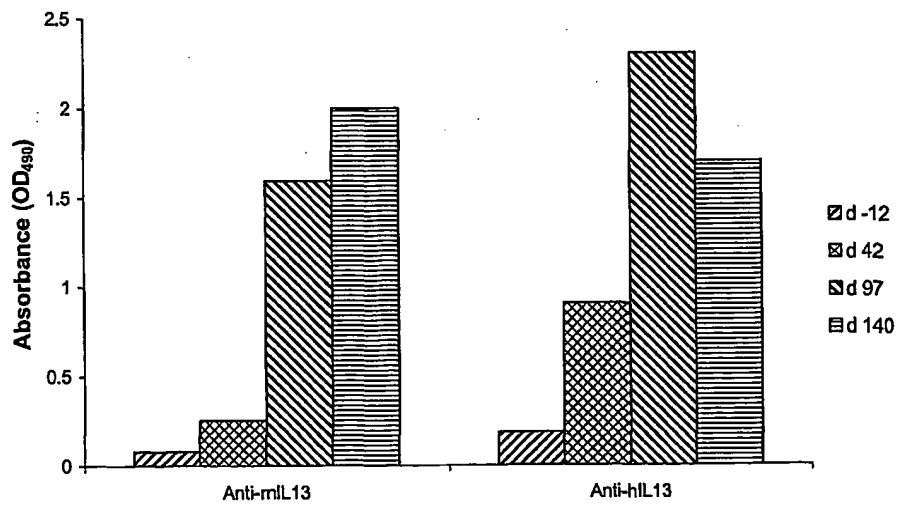


Figure 9

5 ATGGCGCTTTTGGTTGACCACGGTCATTGCTCTCACTTGCCTTGGCGGCTTTGCCTCCCCA 60
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 M A L L L T T V I A L T C L G G F A S P

10 GGCCCTGTGCCTCCCTCTACAGCCCTTAAGGAGCTTATTGAGGAGCTGAGCAACATCACC 120
 61 -----+-----+-----+-----+-----+-----+-----+-----+-----+-----+
 G P V P P S T A L K E L I E E L S N I T

15 CAGAACCAGAAGGCTCCGCTCTGCAATGGCAGCATGGTTTGGAGCATCAACCTGACAGCT 180
 121 -----+-----+-----+-----+-----+-----+-----+-----+-----+-----+
 Q N Q K A P L C N G S M V W S I N L T A

20 GGCATGTTCTGTGTAGCCCTGGATTCCCTGATCAACGTGTCAGGCTGCAGTGCCATCTAC 240
 181 -----+-----+-----+-----+-----+-----+-----+-----+-----+-----+
 G M F C V A L D S L I N V S G C S A I Y

25 AGGACCCAGAGGATATTGCATGGCTTCTGCCCGCACAAGGTCTCAGCTGGGCAGTTTTCC 300
 241 -----+-----+-----+-----+-----+-----+-----+-----+-----+-----+
 R T Q R I L H G F C P H K V S A G Q F S

30 AGCTTGCATGTCCGAGACACCAAAATCGAAGTAGCCCACTTTATAACAAAAGTCTCTTA 360
 301 -----+-----+-----+-----+-----+-----+-----+-----+-----+-----+
 S L H V R D T K I E V A H F I T K L L L

35 CATTTAAAGAACTTTTTTCGCGAGGGACGGTTCAACTGA 399
 361 -----+-----+-----+-----+-----+-----+-----+-----+-----+-----+
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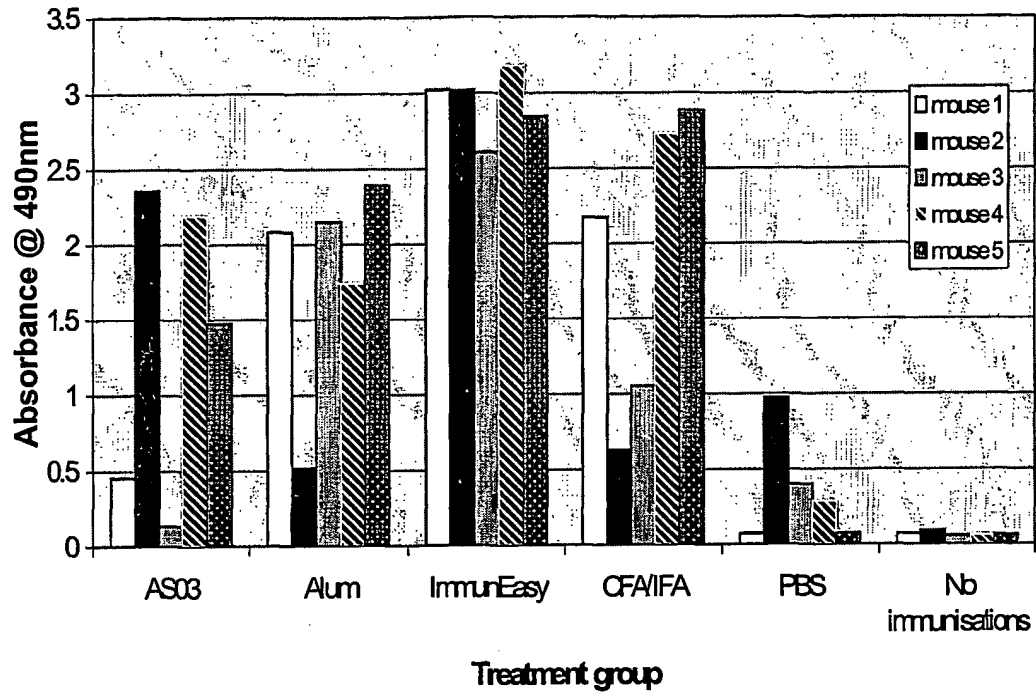
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50

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Figure 10



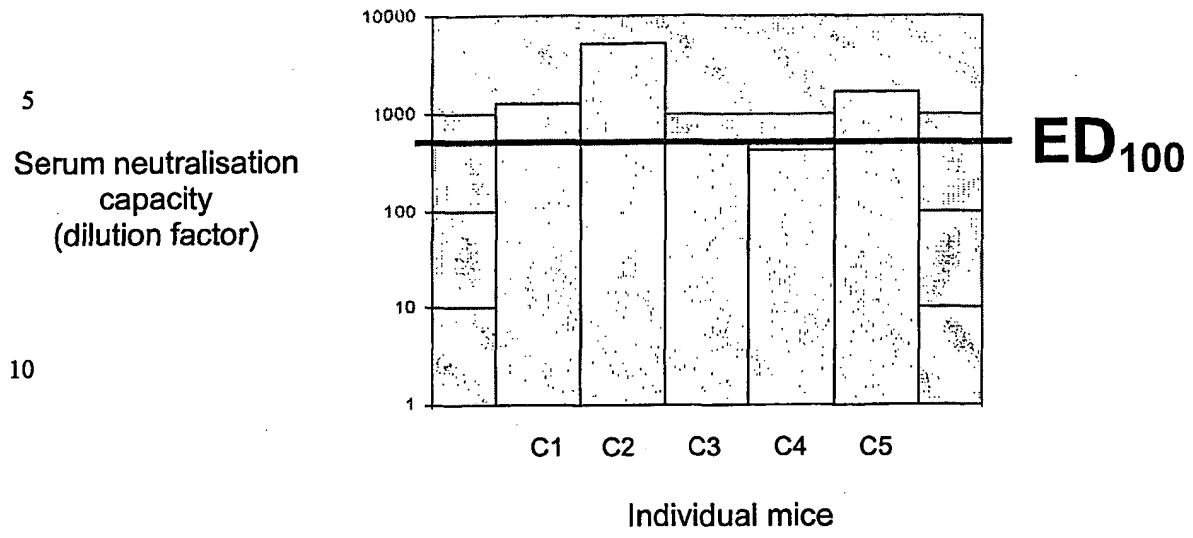
5

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11/13

Figure 11



5
Serum neutralisation
capacity
(dilution factor)

10

15

20

25

30

Figure 12

```

                    atggcgctctgggtgactgcagtcctggctcttgccttggtggtctcgccgcccc
5      1  -----+-----+-----+-----+-----+-----+-----+ 60
-19  M A L W V T A V L A L A C L G G L A A P 0

                    GGGCCGGTGCCAAGATCTGTGTCTCTCCCTGTGACCCTTAAGGAGCTTATTGAGGAGCTG
10     61 -----+-----+-----+-----+-----+-----+-----+ 120
1    1  G P V P R S V S L P V T L K E L I E E L 20

                    ACCAACATCACACAAGACCAGACTCCCCTGTGCAACGGCAGCATGGTATGGAGTGTGGAC
15    121 -----+-----+-----+-----+-----+-----+-----+ 180
21   T N I T Q D Q T P L C N G S M V W S V D 40

                    CTGGCCGCTGGCGGGTTCTGTGTAGCCCTGGATTCCCTGACCAACATCTCCAATTGCAAT
181   -----+-----+-----+-----+-----+-----+-----+ 240
41   L A A G G F C V A L D S L T N I S N C N 60

                    GCCATCTTCAGGACCCAGAGGATATTGCATGCCCTCTGTAACCGCAAGCCCCCACTACG
20    241 -----+-----+-----+-----+-----+-----+-----+ 300
61   A I F R T Q R I L H A L C N R K A P T T 80

                    GTCTCCAGCCTCCCCGATACCAAATCGAAGTAGCCCACTTTATAACAAAAGTCTCACC
25    301 -----+-----+-----+-----+-----+-----+-----+ 360
81   V S S L P D T K I E V A H F I T K L L T 100

                    TACACAAAGAACCTGTTTCGCCGCGGCCCTTCTAA
30    361 -----+-----+-----+-----+-----+-----+-----+ 396
101  Y T K N L F R R G P F * 112

```

35

40

Figure 13

5	-23	----+-----+-----+ MGLTSQLLPPLFFLLACAGNFVHG	0
	1	-----+-----+----- HKCD <u>KNHLREIIGILNEVTGEKTL</u>	24
10	25	----+-----+----- CTELTVTDIFAASKNT <u>TESELVCR</u>	48
	49	-+-----+-----+-- <u>ASKVLRIFYLKHEKDTRCLGATAK</u>	72
15	73	-----+-----+----- <u>NSSVLMELQRLFRAFRCLDGLNSC</u>	96
20	97	---+-----+-----+ PVKEANQSS <u>LKDFLESLSIMQMD</u>	120
	121	----- 126 <u>YSKCSS</u>	
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 Thr Gln Arg Met Leu Gly Gly Leu Cys Asn Arg Lys Ala Pro Thr Thr
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 35 40 45
 Asn Gly Ser Met Val Trp Ser Ile Asn Leu Thr Ala Gly Met Phe Cys
 50 55 60
 Val Ala Leu Asp Ser Leu Ile Asn Val Ser Gly Cys Ser Ala Ile Tyr
 65 70 75 80
 Arg Thr Gln Arg Ile Leu His Gly Phe Cys Pro His Lys Val Ser Ala
 85 90 95

Gly Gln Phe Ser Ser Leu His Val Arg Asp Thr Lys Ile Glu Val Ala
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			20					25					30		
Arg	Glu	Ile	Ile	Gly	Ile	Leu	Asn	Glu	Val	Thr	Gly	Glu	Lys	Thr	Leu
		35					40					45			
Cys	Thr	Glu	Leu	Thr	Val	Thr	Asp	Ile	Phe	Ala	Ala	Ser	Lys	Asn	Thr
	50					55					60				
Thr	Glu	Ser	Glu	Leu	Val	Cys	Arg	Ala	Ser	Lys	Val	Leu	Arg	Ile	Phe
65					70					75				80	
Tyr	Leu	Lys	His	Glu	Lys	Asp	Thr	Arg	Cys	Leu	Gly	Ala	Thr	Ala	Lys
				85				90						95	
Asn	Ser	Ser	Val	Leu	Met	Glu	Leu	Gln	Arg	Leu	Phe	Arg	Ala	Phe	Arg
			100					105						110	
Cys	Leu	Asp	Gly	Leu	Asn	Ser	Cys	Pro	Val	Lys	Glu	Ala	Asn	Gln	Ser
		115					120					125			
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	130					135					140				
Tyr	Ser	Lys	Cys	Ser	Ser										
145					150										

7

INTERNATIONAL SEARCH REPORT

International Application No

PCT/GB 02/00900

A. CLASSIFICATION OF SUBJECT MATTER IPC 7 C12N15/24 C07K14/47 C07K14/54 A61K39/00		
According to International Patent Classification (IPC) or to both national classification and IPC		
B. FIELDS SEARCHED		
Minimum documentation searched (classification system followed by classification symbols) IPC 7 C12N C07K		
Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched		
Electronic data base consulted during the international search (name of data base and, where practical, search terms used) EPO-Internal, BIOSIS, BIOTECHNOLOGY ABS, CHEM ABS Data, SEQUENCE SEARCH, EMBASE, LIFESCIENCES, MEDLINE, PAJ, SCISEARCH, WPI Data		
C. DOCUMENTS CONSIDERED TO BE RELEVANT		
Category *	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	WO 96 04306 A (SCHERING CORP) 15 February 1996 (1996-02-15) the whole document claim 12	6-18, 21-24
X	MCKENZIE A N J ET AL: "Structural comparison and chromosomal localization of the human and mouse iL-13 genes" JOURNAL OF IMMUNOLOGY, THE WILLIAMS AND WILKINS CO. BALTIMORE, US, vol. 150, no. 12, 15 June 1993 (1993-06-15), pages 5436-5444, XP002176473 ISSN: 0022-1767 figure 2	6-16
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<input checked="" type="checkbox"/> Further documents are listed in the continuation of box C.	<input checked="" type="checkbox"/> Patent family members are listed in annex.	
* Special categories of cited documents :		
A document defining the general state of the art which is not considered to be of particular relevance *E* earlier document but published on or after the international filing date *L* document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified) *O* document referring to an oral disclosure, use, exhibition or other means *P* document published prior to the international filing date but later than the priority date claimed	*T* later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention *X* document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone *Y* document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art. *&* document member of the same patent family	
Date of the actual completion of the international search <p style="text-align: center;">14 May 2002</p>	Date of mailing of the international search report <p style="text-align: center;">04/06/2002</p>	
Name and mailing address of the ISA European Patent Office, P.B. 5818 Patentlaan 2 NL - 2280 HV Rijswijk Tel. (+31-70) 340-2040, Tx. 31 651 epo nl, Fax: (+31-70) 340-3016	Authorized officer <p style="text-align: center;">Pilat, D</p>	

INTERNATIONAL SEARCH REPORT

International Application No
PCT/GB 02/00900

C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT

Category °	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	BUITKAMP JOHANNES ET AL: "The cattle interleukin-13 gene: Genomic organization, chromosomal location, and evolution of the promoter." IMMUNOGENETICS, vol. 49, no. 10, 1999, pages 872-878, XP002198871 ISSN: 0093-7711 the whole document	6-11, 13-18, 21-24
X	HEINZMANN A ET AL: "Genetic variants of IL-13 signalling and human asthma and atopy." HUMAN MOLECULAR GENETICS, vol. 9, no. 4, 1 March 2000 (2000-03-01), pages 549-559, XP002198872 ISSN: 0964-6906 the whole document	6-8, 13-18, 21-24
X	EP 0 759 468 A (VIRBAC LAB) 26 February 1997 (1997-02-26)	6-8, 13-18, 21
Y	claims; figure 2 page 6, line 11 - line 15	19, 20
Y	WO 96 02555 A (UNIV IOWA RES FOUND) 1 February 1996 (1996-02-01) cited in the application the whole document	19, 20
X	WO 93 10235 A (SEBALD WALTER) 27 May 1993 (1993-05-27) claims	6-8, 13-18, 21
X	EP 0 911 401 A (BAYER AG) 28 April 1999 (1999-04-28) claims; tables 1, 2	6-8, 13-18, 21
X	WO 00 78334 A (LIM SIEW PHENG ; TAN YIN HWEE (SG); INST OF MOLECUL & CELL BIOLOGY) 28 December 2000 (2000-12-28) the whole document	6-8, 13-18, 21
X	WO 96 04388 A (BROWNE MICHAEL JOSEPH ; CHAPMAN CONRAD GERALD (GB); CLINKENBEARD HE) 15 February 1996 (1996-02-15) claims; examples	6-8, 13-18, 21
A	WO 97 40149 A (BOOTS ANNA MARIA HELENA ; AKZO NOBEL NV (NL); BOS EBO SYBREN (NL);) 30 October 1997 (1997-10-30) the whole document	6-8, 13-18, 21
	-/--	

INTERNATIONAL SEARCH REPORT

International Application No

PCT/GB 02/00900

C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT		
Category °	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
A	WO 99 46992 A (GREGORY RICHARD J ;GENZYME CORP (US); KAPLAN JOHANNE (US)) 23 September 1999 (1999-09-23) page 3, line 6 - line 10; claims page 8, line 10 - line 18 -----	6-8, 13-18
A	WO 96 26218 A (RUDY GEORGE ;HONEYMAN MARGO (AU); LEW ANDREW (AU); AMRAD OPERATION) 29 August 1996 (1996-08-29) page 2; claims; figure 1 -----	6-8, 13-18
A	EP 1 026 238 A (UNIV CALIFORNIA) 9 August 2000 (2000-08-09) page 3, paragraph 9; claims; figure 4 -----	6-8, 13-18
P,X	WO 01 34645 A (JOSHI BHARAT H ;OSHIMA YASUO (US); PURI RAJ K (US); GOVERNMENT OF) 17 May 2001 (2001-05-17) the whole document figure 1 -----	6-18, 21-24

FURTHER INFORMATION CONTINUED FROM PCT/ISA/ 210

Continuation of Box I.2

Claims Nos.: 1-5

Present claims 1-5 relate to an extremely large number of possible compounds. In fact, the claims contain so many variables that a lack of clarity (and/or conciseness) within the meaning of Article 6 PCT arises to such an extent as to render a meaningful search of the claims impossible. Consequently, the search has been carried out for those parts of the application which do appear to be clear (and/or concise), namely compounds wherein the human protein or B cell epitope is derived from cytokine as claimed in claims 6-8, mutated interleukin 13 as claimed in claims 9-12 and subject-matter as claimed in claims 13-25 referring directly or indirectly thereto.

The applicant's attention is drawn to the fact that claims, or parts of claims, relating to inventions in respect of which no international search report has been established need not be the subject of an international preliminary examination (Rule 66.1(e) PCT). The applicant is advised that the EPO policy when acting as an International Preliminary Examining Authority is normally not to carry out a preliminary examination on matter which has not been searched. This is the case irrespective of whether or not the claims are amended following receipt of the search report or during any Chapter II procedure.

INTERNATIONAL SEARCH REPORT

Information on patent family members

International Application No

PCT/GB 02/00900

Patent document cited in search report		Publication date	Patent family member(s)	Publication date
WO 9604306	A	15-02-1996	US 5696234 A	09-12-1997
			AU 3196195 A	04-03-1996
			WO 9604306 A2	15-02-1996
EP 0759468	A	26-02-1997	EP 0759468 A1	26-02-1997
			JP 9118696 A	06-05-1997
WO 9602555	A	01-02-1996	AU 713040 B2	18-11-1999
			AU 1912795 A	16-02-1996
			CA 2194761 A1	01-02-1996
			EP 1167377 A2	02-01-2002
			EP 1167378 A2	02-01-2002
			EP 1167379 A2	02-01-2002
			EP 0772619 A1	14-05-1997
			JP 10506265 T	23-06-1998
			WO 9602555 A1	01-02-1996
			US 6194388 B1	27-02-2001
			US 6207646 B1	27-03-2001
			WO 9310235	A
AT 163196 T	15-02-1998			
AU 671960 B2	19-09-1996			
AU 2928292 A	15-06-1993			
CA 2123315 A1	27-05-1993			
CZ 9401185 A3	15-12-1994			
DE 59209196 D1	19-03-1998			
DK 613499 T3	23-09-1998			
WO 9310235 A1	27-05-1993			
EP 0613499 A1	07-09-1994			
ES 2112340 T3	01-04-1998			
GR 3026600 T3	31-07-1998			
HU 66826 A2	30-01-1995			
JP 7501522 T	16-02-1995			
KR 276410 B1	15-12-2000			
NO 941681 A	06-05-1994			
SK 55494 A3	07-12-1994			
US 5723118 A	03-03-1998			
EP 0911401	A	28-04-1999	EP 0911401 A1	28-04-1999
			AU 9749598 A	10-05-1999
			WO 9920765 A1	29-04-1999
			EP 1023446 A1	02-08-2000
			JP 2001520040 T	30-10-2001
ZA 9809540 A	22-04-1999			
WO 0078334	A	28-12-2000	AU 5617900 A	09-01-2001
			WO 0078334 A1	28-12-2000
WO 9604388	A	15-02-1996	AU 2140699 A	03-06-1999
			AU 3382595 A	04-03-1996
			BR 9508469 A	16-09-1997
			CA 2196200 A1	15-02-1996
			CN 1164872 A	12-11-1997
			CZ 9700256 A3	17-09-1997
			WO 9604388 A1	15-02-1996
			EP 0770135 A1	02-05-1997
			HU 76369 A2	28-08-1997
			JP 10503371 T	31-03-1998

INTERNATIONAL SEARCH REPORT

Information on patent family members

International Application No

PCT/GB 02/00900

Patent document cited in search report		Publication date	Patent family member(s)	Publication date
WO 9604388	A		NO 970374 A	19-02-1997
			NZ 292124 A	28-10-1998
			PL 318380 A1	09-06-1997
			US 5783181 A	21-07-1998
			ZA 9506253 A	08-01-1997
WO 9740149	A	30-10-1997	US 5843449 A	01-12-1998
			AU 724547 B2	28-09-2000
			AU 2386997 A	12-11-1997
			BR 9708714 A	03-08-1999
			CA 2251584 A1	30-10-1997
			CN 1216582 A	12-05-1999
			CZ 9803333 A3	17-03-1999
			WO 9740149 A1	30-10-1997
			EP 0904369 A1	31-03-1999
			JP 2000509265 T	25-07-2000
			NO 984835 A	16-12-1998
			NZ 332311 A	26-05-2000
			PL 329350 A1	29-03-1999
			TR 9802082 T2	21-09-2000
WO 9946992	A	23-09-1999	AU 3102999 A	11-10-1999
			CA 2322624 A1	23-09-1999
			EP 1071333 A1	31-01-2001
			JP 2002506618 T	05-03-2002
			WO 9946992 A1	23-09-1999
WO 9626218	A	29-08-1996	AU 706774 B2	24-06-1999
			AU 4659796 A	11-09-1996
			WO 9626218 A1	29-08-1996
			CA 2213301 A1	29-08-1996
			EP 0815136 A1	07-01-1998
			JP 11500719 T	19-01-1999
EP 1026238	A	09-08-2000	EP 1026238 A2	09-08-2000
			AT 207537 T	15-11-2001
			AU 661684 B2	03-08-1995
			AU 1516392 A	24-12-1992
			CA 2070004 A1	19-12-1992
			DE 69232147 D1	29-11-2001
			DK 519469 T3	10-12-2001
			EP 0519469 A1	23-12-1992
			ES 2165350 T3	16-03-2002
			JP 7070182 A	14-03-1995
			KR 262247 B1	15-07-2000
			US 5674978 A	07-10-1997
			US 5475086 A	12-12-1995
			US 6011139 A	04-01-2000
			US 5846740 A	08-12-1998
			US 5705626 A	06-01-1998
			US 5998366 A	07-12-1999
WO 0134645	A	17-05-2001	AU 1599301 A	06-06-2001
			WO 0134645 A2	17-05-2001